ABSTRACTS COLLECTION

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Presenting author names **are bold** in the contributor lists.

P01 Cancer Genetics

P01.001.A Human synonymous codon variation is constrained within multivalent coding regions (Award Candidate)

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Background: Cellular homeostasis hinges on precise protein dosage control, particularly for proteins harbouring disordered low complexity domains (LCDs). We recently reported a new regulatory mechanism termed 'interstasis', facilitating mutual dosage regulation of LCD-containing proteins. Interstasis leverages multivalent CDS regions encoding LCDs, which stems from the repetitive amino acid arrangement, similarity of codons for biophysically akin amino acids, and conserved synonymous codon biases. Specific RNA-binding proteins bind to multivalent RNA regions and orchestrate the interstasis through feedback regulation of mRNAs encoding LCD-containing proteins.

Materials and methods: Exploring the impact of RNA multivalency on human genetic variation in coding regions, we analysed common polymorphisms from dbSNP. Additionally, we similarly explored somatic variation in cancer using data from Cosmic.

Results: For germline human variation, we found that the more multivalent a CDS region is, the greater the relative synonymous constraint - i.e. the less variation is tolerated in synonymous relative to non-synonymous variation. In cases such as arginine that contains 6 possible codons, human synonymous variation tends to maintain high RNA multivalency by mimicking the codons of other nearby amino acids.For somatic variation in cancer, multivalent regions exhibited increased relative synonymous variation in Cosmic as compared to dbSNP, and we identified transcripts with particularly high rates of multivalence-disrupting synonymous variants as candidates for further study.

Conclusion: Our findings underscore the importance of synonymous constraint patterns linked to RNA multivalency in LCDs. This opens new opportunities for future investigations to explore the contribution of these constraints for the interstasis-mediated dosage control of LCD-containing proteins.

C.O.I. Disclosure:

R. Dore: None. **J. Ule:** None.

P01.002.B A germline and somatic mutation sorting (GeMSort) algorithm for extracting presumed germline pathogenic variants in liquid genomic profiling: insights from C-CAT database

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Background: Liquid biopsy comprehensive genomic profiling (CGP) testing is performed on circulating tumor DNA to identify somatic variants with the aim of determining therapeutic strategies. Pathogenic variants of germline origin in genes

associated with hereditary tumor syndromes can be simultaneously detected using this test. Nonetheless, it is often challenging to differentiate whether the variants are of somatic or germline origin. The differentiation criteria were primarily based on the allele frequencies of the variants (VAF). However, more evidence is needed to establish clear criteria, and it is often difficult to differentiate the origin of variants based on VAF alone.

Material and methods: In this study, using the national database of the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), which accumulates real-world data on CGP testing in Japan, we analyzed 169,370 variants detected in 11,399 patients registered with FoundationOne Liquid CDx testing.

Results: By extracting the predominantly presumed somatic and germline variants, we established a criterion for VAF that could achieve high specificity and sensitivity. Further investigation into the detection status of other variants led to the development of an algorithm for differentiating somatic/germline variants of genes associated with hereditary tumor syndromes. Based on this algorithm, 726 variants were extracted as presumed germline pathogenic variants among the genes with high germline conversion rates in 710 patients.

Conclusion: This algorithm should help to discriminate with high accuracy whether the variants detected in liquid CGP tests are of somatic/germline origin, although further analyses are required to confirm the validity of this algorithm.

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P01.003.C STAG2 as a new player in medulloblastoma: in vitro and in vivo models

Antonella Lettieri, *Silvia Castiglioni, Simona Totaro, Silvia Rasetti, Elisabetta Di Fede, Chiara Parodi, Paolo Grazioli, Elisa A. Colombo, Esi Taci, Thomas Vaccari, Cristina CG Gervasini, Valentina Massa*

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Background: Medulloblastoma (MB) is a cerebellar embryonic tumor, probably due to neural progenitor impaired differentiation. However, the underlying genetics and predisposing factors are still unclear. The cohesin complex role in cerebellum development, gene expression regulation, and DNA signaling repair suggest this structure as a possible oncogenic candidate. Among cohesins, STAG2 is the most frequently affected in cancers, and its loss of function is believed to promote tumorigenesis by altering DNA architecture.

Material and methods: We studied the impact of *STAG2* silencing on cell functioning and DNA damage in 2D and cell spheroid models. Then, we exploited 3D cerebellum organoids to study the effect of *STAG2* deficiency on its development. Last, the in vivo fly model obtained by *SA1* silencing, the ortholog of *STAG2*, was characterized to assess tumor-related phenotypic features by investigating neural precursors differentiation and flies' viability.

Results: In vitro, we highlighted that: (1) *STAG2*-deficient cells present increased DNA damage and (2) *STAG2*-silenced cerebellum organoids exhibit a deregulated expression of genes related to cerebellar development. In vivo, we showed a defective differentiation of neuroblasts during larvae development and mass formation within adult fly brains with a lowered life expectancy consistent with the malignant phenotype.

Conclusion: These models not only aim at unveiling the contribution of STAG2 in promoting MB tumorigenesis but will also represent a basis for testing possible targeted therapies, such as PARP inhibitors, recently implemented in trials for tumors carrying cohesin variants and shown to be more effective in cases with altered DNA signaling repair.

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P01.004.D Identification of methylation-based Wilms tumour signatures to recognise children with underlying genetic predisposition

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Background: Recognising cancer predisposition in paediatric oncology is crucial, as it may impact diagnosis, treatment, and surveillance. Approximately 10% of childhood cancers have an underlying predisposition, reaching up to 30% in Wilms tumours (WT). Identifying predispositions remains challenging due to the large number of (epi)genetic factors, phenotypic variability, and potential mosaicism. Tumours sharing a genetic predisposition often exhibit common somatic signatures, including DNA methylation patterns. We hypothesise that these somatic molecular patterns can identify specific types of underlying predisposition, independent of phenotypic presentation.

Material and methods: We performed unsupervised hierarchical clustering of DNA methylation data in a cohort of 139 WT patients with known predispositions. For 121 patients, matching RNA-seq data were available, and for 105 patients, whole genome sequencing data were included to correlate molecular patterns with genetic aberrations.

Results: Five distinct methylation subgroups were identified. One subgroup included all samples with pathogenic germline *WT1* variants (7/7, 100%) and most samples with somatic *WT1* aberrations (9/11, 81%), along with additional pathogenic *CTNNB1* variants. Patients diagnosed with Beckwith-Wiedemann spectrum were predominantly distributed across two other subgroups. These findings suggest that specific WT subtypes are associated with distinct molecular and genetic features, which can aid in preselecting cases for genetic referral.

Conclusion: Our findings provide proof-of-principle for a tumour-first approach using methylation data to identify WT predispositions. Future efforts will focus on developing a classifier based on these insights for integration into routine diagnostics, enabling efficient identification of patients with a predisposition for WT development.

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P01.005.A Comparative analysis of *BRCA* pathogenic variant testing: first nation-wide population screening program versus genetic counseling

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Background: Israel recently initiated the first nation-wide population screening (PS) program for *BRCA* pathogenic variants (PVs), which is expected to reduce morbidity and mortality. However, implementation presents unique challenges. We aimed to compare characteristics, risk-reducing actions and psychosocial outcomes of PS vs. carriers identified following onco-genetic counselling (GC).

Materials and methods: All healthy *BRCA1/2* PV carriers identified 2021–2024 attending the high-risk surveillance-clinic in Shaare-Zedek Medical-Center; data collected from medical records and psychosocial and knowledge-assessment questionnaires.

Results: Women were referred to PS by gynecologists (41.7%), breast-surgeons (17.7%) or family-practitioners (6.2%). PS group included 202 women; GC group 150. Table compares characteristics, risk-reducing measures, and psychosocial outcomes. Mean age (37.6yrs ± 11) and college-level education (82.8%) were comparable between groups. PS carriers were less likely to have cancer family history, (30.4% vs. 72.7% $p < 0.001$), and more likely to have a *BRCA2* (vs. *BRCA1*) PV than GC (62.9% vs. 48.7%, $p = 0.01$). Risk-reducing-salpingo-oophorectomy (RRSO) rates at the recommended age were similar between groups, in PS carriers: *BRCA1* ≥ 40yrs: 79.2%, *BRCA2* ≥ 45yrs: 81.4%. Both groups reported high general satisfaction, but PS reported slightly lower satisfaction with pretest information, delivery of results, and health decision. Groups had similar perceived control (PPC), distress (IES) anxiety (STAI), and knowledge scores. In multivariate-analysis, there were no significant differences in psychosocial outcomes.

Conclusion: This first nation-wide *BRCA*-PS resulted in actionable measures with high uptake of RRSO and demonstrates favorable psychosocial outcomes. The data provides support for PS, but refinement and long-term evaluation are necessary.

	Population screening $n = 202$ *n* (%) or (mean ± SD)	**Genetic counseling $n = 150$ *n* (%) or (mean ± SD)**	**Total $n = 352$ n (%) or (mean ± SD)**	***p*-value**
Sociodemographic characteristics				
Age at testing	38.4 ± 10.9	36.6 ± 11.2	37.6 ± 11.1	0.15
College education	82/99 (82.8%)	67/81 (82.71%)	149/180 (82.8%)	1
Married/In partnership	90/102 (88.2%)	69/84 (82.14%)	159/186 (85.5%)	0.29
Children (any)	93/112 (83%)	75/101 (74.3%)	168/213 (78.9%)	0.13
Breast/ovarian cancer family history (any)	49/161 (30.4%)	99/136 (72.7%)	148/297 (49.8%)	**<0.001**
BRCA1 pathogenic variant	75/202 (37.1%)	77/150 (51.3%)	152/352 (43.2%)	**0.01**
BRCA2 pathogenic variant	127/202 (62.9%)	73/150 (48.7%)	200/352 (56.8%)	**0.01**
Risk reducing salpingo-oophorectomy (RRSO)	62/197 (31.47%)	40/147 (27.2%)	102/343 29.4%	0.47
Age at RRSO	50.05 ± 7.63	49.07 ± 8.34	49.66 ± 7.99	0.53
Months from test to RRSO	6.9 ± 7.0	9.6 ± 9	8.04 ± 8.0	0.11
RRSO ≥40 for *BRCA1*	20/27 (79.2%)	18/21 (85.7%)	38/48 (79.2%)	0.48
RRSO ≥45 for *BRCA2*	35/43 (81.4%)	14/22 (63.5%)	49/65 (75.4%)	0.14
Psychosocial outcomes				
Satisfaction (score 1–5):				
General	3.8 ± 1.0	4.02 ± 1.1		0.06
With pretest information	3.61 ± 1.2	3.90 ± 1.2		**0.045**
With delivery of results	3.34 ± 1.3	3.89 ± 1.2		**0.001**
With post-test counseling	3.68 ± 1.1	3.90 ± 1.2		0.09
With Health Decision (score 6–30)	24.61 ± 3.9	25.9 ± 3.6		**0.02**
Perceived personal control (score 0–2)	1.39 ± 0.49	1.43 ± 0.51		0.59
Impact of Events Scale (score 0–75)	16.41 ± 15.7	13.18 ± 16.2		0.15
State Trait Anxiety Inventory (score 6–24)	14.41 ± 2.0	14.71 ± 2		0.49
Knowledge (score 0–10)	8.72 ± 1.1	8.78 ± 1.1		0.7

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P01.006.B Optical genome mapping in hematological malignancies: is it time for conventional cytogenetic approaches to stand aside?

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Background: Chromosomal aberrations characterization in onco-hematology is performed by Conventional Cytogenetic Techniques (CCT), often sequentially required to complete the diagnostic workflow. Optical Genome Mapping (OGM) is an innovative technology that analyzes ultra-high molecular weight DNA to detect chromosomal aberrations with high resolution. Our study evaluates OGM application in hematological malignancies diagnostic routine.

Material and methods: We analyzed 18 samples from patients with hematological malignancies, including 10 chronic lymphocytic leukemia (CLL), seven acute myeloid leukemia (AML), and one chronic myeloid leukemia (CML).

Results: Using SaphyrTM, OGM analysis takes 4–6 working days for six samples. Twelve samples completed the study, while six samples failed during the process. OGM analysis confirmed the presence of all the abnormalities (aneuploidies, deletions and translocations) found using CCT. In addition, OGM made it possible to characterize abnormalities underrepresented in the sample (VAF 6%), to define the genetic origin of additional chromosomal material, and to detect complex abnormalities such as marker chromosomes and chromothripsis events.

Conclusion: Our data show complete concordance between OGM and CCT results. Since CCT includes karyotype and FISH panels for CLL, OGM is cost-effective regarding analysis time and operator dependency. However, for AML and CML at the onset, OGM may not be cost-effective because it is not compliant with guideline reporting requirements. OGM has the potential to improve the detection of chromosomal abnormalities, making it particularly suitable for the characterization of hematologic malignancies. Further standardization and automation of OGM protocols are essential to make them applicable to diagnostic routines.

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P01.007.C Multi-locus inherited neoplasia allele syndrome (MINAS): prevalence and phenotype in the German Consortium of Hereditary Breast and Ovarian Cancer

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Background: Next generation sequencing in families with hereditary breast and ovarian cancer (HBOC) has increased the detection of individuals with a MINAS. However, data on triple-heterozygous (TH) pathogenic variant (PV) carriers remain limited. Large registry studies are necessary to determine genotype-phenotype correlations, assess cancer risks, and develop personalized prevention recommendations for MINAS.

Materials and methods: We searched the database of the German Consortium (GC) of HBOC for individuals with a co-occurrence of two or more PVs in the GC-HBOC gene panel that comprises cancer predisposition genes for HBOC and candidate genes. We analyzed the genotypes and phenotypes of index patients and family members.

Results: In a cohort of over 26,000 PV carriers, we identified 409 individuals with two or more PVs. Among these, twelve individuals from seven families carried three PVs. All TH individuals carried PVs in at least one of the genes *BRCA1*, *BRCA2*, or *CHEK2*, in part combined with PVs in other genes, reflecting the prevalence of single heterozygotes in the database. Genotypes and phenotypes of TH individuals are shown in Table 1.

Conclusion: In summary, the database proved to be a valuable tool for genotype-phenotype correlation. Critical evaluation of counselees' pedigrees and consideration of multiple PVs is crucial, especially when cancer cases occur in both hereditary lines. Future studies, in which both index persons and their family members are examined using gene panels, may enable comprehensive identification of individuals with MINAS, leading to more personalized prevention methods.

Genotype and phenotype of TH individuals. BC breast cancer, OC ovarian cancer, incl. candidate genes

Family	Gene 1	Gene 2 including candidate gene *NBN*	Gene 3 including candidate gene *FANCM*	Sex	Index	Phenotype of TH individual (age in years)
I	*BRCA1*	*BRCA2*	*CHEK2*	female	yes	bilateral BC (56, 69)
II	*BRCA1*	*BRCA2*	*CHEK2*	male	yes	pancreatic cancer (34)
III	*BRCA1*	*BRCA2*	*PTEN*	female	yes	bilateral BC (35, 54)
IV	*BRCA1*	*NBN*	*FANCM*	female	yes	BC, OC, pancreatic cancer (72, 72, 72)
IV	*BRCA1*	*NBN*	*FANCM*	female	no	OC (58)
IV	*BRCA1*	*NBN*	*FANCM*	male	no	cancer-free (61)
IV	*BRCA1*	*NBN*	*FANCM*	female	no	cancer-free (23)
V	*ATM*	*BRCA2*	*CHEK2*	female	yes	BC (40)
V	*ATM*	*BRCA2*	*CHEK2*	female	no	BC (65)
V	*ATM*	*BRCA2*	*CHEK2*	female	no	BC (72)
VI	*ATM*	*CHEK2*	*PMS2*	female	yes	cancer-free (61)
VII	*BRCA2*	*CHEK2*	*RAD51C*	female	yes	BC (34)

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P01.009.A Integrating systematic RNA analysis in routine diagnosis of cancer predisposition with SEALigHTS: a cost-effective, high-throughput RNA splicing analysis

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Background: Diagnostic analysis of mRNA is essential, as altered splicing is a frequent cause of genetic diseases. High throughput splicing studies remain challenging to implement in routine diagnostics. To address this, we developed SEALigHTS (splice and expression analyses by ligation and high throughput sequencing), a rapid and cost-effective RT-MLPA-derived technique applicable on easily accessible samples for simultaneous panel RNA analysis of multiple patients, using probes designed at exon extremities.

Material and methods: After reverse transcription and probing on cDNA, neighbouring probes are ligated, sequenced by NGS and quantified using unique molecular identifiers. We designed a panel covering 42 genes (2,195 probes) implicated in breast and/or ovarian, colorectal cancer and polyposis predispositions. We studied 37 highly evocative patients and initiated the multicenter prospective project STRATEGIC, designed to include 1,000 consecutive patients suspected of a cancer predisposition. This protocol aims to evaluate the added value of systematically combining RNA and DNA analyses in routine practices.

Results: Following a training phase on 40 samples, SEALigHTS was validated in another laboratory on 56 samples carrying splicing variants previously characterized by RNAseq, achieving 96% sensitivity and 94% specificity. Preliminary results in highly evocative and the first 120 STRATEGIC patients underscore the significant contribution of RNA analysis with 7 new diagnoses total, including detection of deep intronic variants, reclassification of a duplication, identification of splicing variants poorly predicted by in silico tools.

Conclusion: These findings highlight the potential of SEALigHTS to enhance genetic diagnostics and improve our knowledge on variant typically overlooked at the RNA level.

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P01.010.B Homologous recombination deficiency is uncommon in the cancers from children with heterozygous germline pathogenic *BRCA1*, *BRCA2* or *PALB2* variants

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Background: In 5–15% of pediatric cancer cases, a germline pathogenic variant (gPV) in a cancer predisposing gene can be identified. For some genes, biallelic variants are known to cause pediatric cancer, whereas heterozygous variants are associated with adult cancer. Examples include *BRCA1*, *BRCA2* and *PALB2*, which play a role in homologous recombination (HR). Although previous studies have claimed enrichment of gPVs in *BRCA1* and *BRCA2* in pediatric cancer, it is unclear how often these variants cause tumour initiation.

Material and methods: We analysed indicators of causality, including somatic second hits and mutational footprints associated with HR deficiency (HRD), in whole genome sequencing (WGS) data of pediatric cancers with a heterozygous gPV in *BRCA1*, *BRCA2* or *PALB2* (n = 49), and in 7 cases of *BRCA2*-associated Fanconi anaemia (FA) as positive controls. Additionally, the CHORD classifier was applied, which integrates single base substitutions (SBSs), insertions/deletions (indels) and structural variants into a single HRD prediction. An unselected WGS control cohort of 653 pediatric tumours was included.

Results: In only one osteosarcoma case with a gPV in *BRCA2* did we identify a somatic second hit. SBS signature SBS3 was found in 32% of control cases and thus appeared unsuitable for HRD detection. Indel signature ID6 and CHORD confidently showed signs of HRD in this osteosarcoma and the FA cases, but not in the other samples.

Conclusion: HRD due to a somatic second hit was found in one osteosarcoma but appears to be rare in pediatric cancer patients with heterozygous gPVs in *BRCA1*, *BRCA2* or *PALB2*.

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P01.011.C Cancer risks for *MSH6* pathogenic variant carriers

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Background: Lynch syndrome (LS) is a hereditary cancer syndrome caused by (likely) pathogenic variants (LP/P) in DNA mismatch repair genes, including *MSH6*. It is associated with elevated lifetime risks for colorectal cancer (CRC), endometrial cancer (EC), and other malignancies. However, cancer risks specific to *MSH6*-associated LS, particularly for non-colorectal cancers, remain poorly defined. This study aims to provide refined cancer risk estimates for individuals with *MSH6* LP/P.

Material and methods: We conducted a retrospective cohort study of 360 families with 1118 known *MSH6* LP/P carriers identified in the Netherlands between 1995 and 2020. Pedigree data were collected from multiple clinical centers, and cancer diagnoses were confirmed through medical records. Age- and sex-specific hazard ratios (HRs) and cumulative risks (CRs) were estimated using segregation analysis, appropriately adjusted for ascertainment.

Results: CR by age 80 for *MSH6* LP/P carriers were 36% in males (95% CI:25–48%) and 21% in females (95% CI 13–32%) for CRC, and 23% in females (95% CI:15–43%) for EC. Elevated risks were observed for ovarian cancer (OC) (6.4%, 95% CI:3–14.8%; HR 5.58, p = 0.00037), urinary tract cancers (10.1% in males, 4.1% in females; HR 2.52, p = 0.012), and biliary tract cancers (4.9% in males, 4.2% in females; HR 2.76, p = 0.031). No increased risks were identified for prostate or breast cancer.

Conclusion: This study refines cancer risk estimates for MSH6 LP/P carriers, suggesting the need for delayed CRC screening in males and females and proactive discussions regarding prophylactic surgery for females to address elevated risks for EC and OC.

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P01.012.D Identification of cancer somatic mutations in miRNA genes

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Background: miRNAs are noncoding RNAs that play an important role in cancer, acting as tumor suppressors or oncogenes. Although thousands of somatic mutations, including numerous cancer-driving mutations, have been identified in protein-coding sequences, very little is still known about cancer somatic mutations in miRNA genes. The delay in this area results mainly from the lack of appropriate tools and experimental approaches dedicated to the analysis of noncoding elements, including miRNA genes.

Material and methods: We developed Whole MIRnome Sequencing (WMS), a new Next Generation Sequencing (NGS)-based platform, dedicated to targeted sequencing of all miRNA genes and a panel of genes playing a role in miRNA biogenesis and functioning. We used OncodriveFML to analyze the pattern of somatic mutations and detect signals of positive selection to reveal candidate cancer drivers.

Results: Using WMS, we sequenced, with high coverage (>700x), ~280 cancer samples of different cancer types, including fresh frozen and degraded FFPE samples. We identified 1,272 cancer somatic mutations, whose reliability was confirmed by several alternative methods. Many of the mutations were located in functional regions of cancer-related miRNA genes such as *MIR1*, *MIR101*, *MIR139*, *MIR146B*, and *MIR34C*. We also identified several overmutated miRNAs and one potential miRNA driver gene, *MIR3928*, specifically mutated in basal cell carcinoma (BCC).

Conclusion: WMS is a cost-effective and sample-saving tool for identifying germline and somatic mutations in miRNA genes in different types of samples. Our proof-of-concept experiment demonstrated that WMS may provide data for identifying potential cancer drivers and characterizing miRNA genes of interest.

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P01.013.A Subclass dependent biomarkers involved in uveal melanoma progression, cell plasticity and metastatic risk

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Background: Uveal Melanoma (UM) is an aggressive cancer originating from melanocytes in the uveal tract. Despite extensive research, the survival rate for metastatic UM has not significantly improved. To date, there are at least five subclasses, each with unique survival patterns and genomic profiles. We aimed to identify subclass-specific biomarkers, validate their oncogenic relevance, and evaluate candidates in their spatial context.

Material and methods: Biomarkers were identified in two independent in-house multi-omics datasets and validated in three additional validation cohorts. Cell proliferation, motility, and morphology were investigated after knock-out or overexpression in UM cell lines and dissemination and tumor volume in xenograft zebrafish experiments. We evaluated the spatial context of biomarker expression in high, intermediate, and low-risk whole tumor and tissue microarray slides.

Results: We identified and validated several potential oncogenic biomarkers, including six transcription factors (ELL2, KDM5B, REXO4, COL9A3, RBFOX2, and FOXD1) in high-risk UM and six transcripts (ABHD6, CSRNP1, BTG2, DHX16, and TAGLN) that differentiate between intermediate-high and intermediate-low risk. In-vitro and in-vivo experiments of biomarkers (FOXD1, RBFOX2, COL9A3) show effects on cell morphology, attachment, and proliferation in high-risk cells, increased levels of plasticity markers, reduced tumor volume and cell dissemination and a more aggressive behaviour in vitro and in vivo.

Conclusion: Metastatic risk is influenced by genetic and transcriptional drivers. Our in-vitro and in-vivo experiments underscore the oncogenic relevance of these drivers. We are currently correlating spatial differences and the microenvironment of cells expressing these drivers to better understand the impacted regulatory mechanisms.

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P01.014.B TP53 p.R181H is associated with a distinct phenotype enabling personalized clinical handling (Award Candidate)

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Background: Heritable *TP53* related cancer syndrome, also known as Li-Fraumeni Syndrome (LFS), is a severe cancer predisposition syndrome caused by germline variants in the *TP53* tumor suppressor gene. Due to the broad tumor spectrum, clinical management and cancer surveillance are challenging. Currently, all LFS patients undergo a standardized surveillance program, including whole-body magnetic resonance imaging (MRI), regardless of their specific *TP53* variant. The *TP53* variant p.R181H is particularly prevalent in the Swedish LFS cohort, and carriers may benefit from a more personalized approach to clinical management and surveillance.

Material and methods: We analyzed genomic and clinical data from the Swedish nationwide germline *TP53* database (SWEP53), including 189 individuals from 86 families. Our study assessed cancer incidence, tumor type and survival rates. Additionally, we performed haplotype analysis to investigate whether p.R181H is a founder variant.

Results: The p.R181H variant was present in 22% of all Swedish g*TP53* families (19 of 86) and is a potential Swedish founder. Compared to carriers of other *TP53* variants, individuals with p.R181H had lower cancer incidence ($p < 0.0001$) and longer survival ($p < 0.0001$). Adult female carriers primarily developed breast cancer, with the earliest onset occurring at 29 years old, and no cases of childhood cancers (<18 years) were observed in the cohort.

Conclusion: Carriers of germline *TP53* variant p.R181H could benefit from targeted surveillance with exclusively breast MRI and children should not be tested until adulthood.

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P01.015.C Impact of optical genome mapping in acute leukemia diagnosis - insights following one-year of clinical deployment as a first-line diagnostic test

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Background: With the clinical implementation of optical genome mapping (OGM) as a first-line diagnostic test for newly diagnosed acute leukemias in November 2023, our lab underwent a transformative shift in standard-of-care (SOC) testing. Here, we present our one-year experience with test deployment and prospectively evaluated clinical utility.

Materials and methods: One year following clinical implementation, 158 adults with acute leukemia were profiled by OGM, including 113 AML/high-grade myeloid neoplasms, 34 B-ALL, 7 T-ALL, and 4 mixed phenotype or undifferentiated acute leukemias. Performance metrics were monitored, including quality control (QC) success rate, analytic performance and added clinical yield against parallel karyotype and FISH testing.

Results: We demonstrate 80% (127/158) first-pass and 100% overall QC success rates. Of 248 variants detected by karyotype/FISH, 245 were concordantly detected by OGM (sensitivity 98.8%). In 158 cases, we reported over 483 variants (average ~3/case), including 269 tier-1A/B variants. A reportable finding by OGM was identified in 32% (25/77) cases identified as normal/failed by karyotype, including 40% (10/25) having a tier-1A variant. We

identified 24% (38/158) cases with added clinical yield, whereby a tier-1A/1B/2 finding was not identified by SOC, including 9.5% (15/158) cases with significantly altered classification/diagnoses primarily due to identification of clinically relevant rearrangements.

Conclusion: OGM has demonstrated striking clinical utility, uncovering diagnostically and prognostically significant alterations previously cryptic by low resolution analyses. With extensive planning, thorough training, engagement of clinical partners and conservative retention of parallel karyotype testing, implementation of this novel technology has been feasible and clinically impactful to our patient population.

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P01.016.D Characterisation of copy number variants with a multigene NGS panel in hereditary cancer patients, reveals a distinctive *PALB2* contribution to the diagnostic yield

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Background: The use of Next Generation Sequencing multi-gene panels has led to the identification of numerous genetic variants associated with breast, ovarian, and pancreatic cancer. Despite these advancements, many DNA alterations remain under characterized. Copy Number Variations (CNVs), defined as deletions and duplications of specific DNA regions, account for up to 10% of pathogenic variants and can occur in any gene tested in routine diagnostics. However, CNVs results in cancer-predisposing genes beyond *BRCA1* and *BRCA2*, such as *PALB2*, remain limited.

Material and methods: This study analyzed data from 2949 patients, primarily affected by breast or ovarian cancer, who underwent diagnostic testing with a 22-gene hereditary cancer panel between 2018 and 2023, with a focus on CNVs data.

Results: Aligned with comparison studies a total diagnostic yield of 14.8% was observed, with pathogenic variants in *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, and *PALB2* accounting for over half of the positive findings. In contrast, CNVs were found in 1.4% of patients, displaying a peculiar distribution pattern. *PALB2* exhibited the highest frequency of pathogenic CNVs (66.7%) and these alterations represented 62.2% of all *PALB2* pathogenic variants. Notably, 24/28 *PALB2* CNV carriers shared an identical pathogenic exon 11 deletion. Further investigations revealed common breakpoints and origin among patients, suggesting that geography may have shaped genetic variability in the cohort.

Conclusion: These data provide insights into the relevance of CNVs detection, which can provide valuable information in variant-associated cancer risk assessment and reveal distinctive patterns of variant prevalence and distribution across different regions and ethnic groups.

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P01.017.A Impact of *TET2* mutations on clinicopathological features and therapy response in 889 sporadic colorectal cancers

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Background: TET2 is a DNA demethylase critical for maintaining epigenomic stability and a known tumor suppressor in hematologic malignancies. Colorectal cancer (CRC) is characterized by genomic and epigenomic instability, which drives tumorigenesis. Studies in mice have revealed that *TET2* loss promotes inflammation, a key driver of CRC development. Emerging evidence suggests that *TET2* plays a role in modulating responses to cancer therapies. However, its contribution to CRC pathogenesis and therapeutic outcomes remains poorly defined.

Material and methods: We analyzed 889 primary CRC samples from the AACR GENIE Biopharma Collaborative CRC 2.0 dataset, containing genomic and phenomic data. An in-house computational pipeline identified *TET2* mutations (SNVs and Indels) and evaluated associations with clinicopathological features, co-occurring mutations, pathways, and therapy outcomes. Statistical analyses included Fisher's exact tests (Benjamini-Hochberg correction) and log-rank tests. Pathway enrichment focused on co-occurring mutations.

Results: *TET2* mutations were identified in 6.4% of CRCs, predominantly as missense (81.9%). These mutations were significantly associated with stage II, grade II, right-sided tumors, and MSI-High status (all $p < 0.05$). Co-occurring mutations, such as *POLE* and *POLD1* (adj $p < 0.05$), are usually linked to increased mutational burden, while enrichment in DNA damage response and chromatin remodeling pathways suggests roles in modulating chemosensitivity. Patients with *TET2*-mutated tumors showed significantly improved overall survival compared to wild-type, both with FOLFOX treatment ($p < 0.05$) and without it ($p = 0.021$).

Conclusion: *TET2* mutations are associated with a CRC subset characterized by specific clinicopathological features and favorable survival outcomes. Validation on larger cohorts is ongoing.

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P01.018.B Genetic landscape of prostate cancer in over 1000 patients - a germline and somatic approach

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Background: Prostate cancer (PC) is the most common cancer among males. Somatic loss of function (LoF) variants in tumor-suppressor genes with high allele frequency in prostate tumours reflect, in several cases, a germline origin. Herein, we present the mutation spectra and the prevalence of disease-causing variants in PC patients.

Materials and methods: A total of 509 formalin-fixed paraffin embedded (FFPE) tissues and 500 peripheral blood samples from unselected prostate cancer patients were referred to our center for clinical genetic testing. Libraries were prepared according to the

manufacturer (AmoyDx® HANDLE HRR NGS Panel, Amoy Diagnostics Co.) and sequenced on a NextSeq500 system (Illumina). Bioinformatic analyses were performed using the AmoyDx ANDAS Data Analyzer.

Results: The prevalence of germline pathogenic/likely pathogenic variants was 11% (55/500). The most frequently mutated genes were *BRCA2* (4.8%) and *ATM* (2.2%), followed by *CHEK2* (0.8%), *BRIP1* (0.8%), *BARD1* (0.6%), *PALB2* and *RAD54L* (0.4% respectively). A total of 128/509 (25.2%) tumour samples harboured LoF variants. Consistently, the most commonly mutated genes were *BRCA2* (4.1%) and *ATM* (2.4%). Rare somatic variants were detected with 2.4% total frequency in other genes tested (*CHEK2, PALB2, RAD54L, RAD51C*). Interestingly, *PTEN* clinically significant somatic variants present high prevalence accounting for 2.2% of prostate tumours.

Conclusion: Overall, our data agree that the *BRCA2* gene is the key-player in PC followed by *ATM*. The detection of mutations in other genes such as *CHEK2, PALB2* and *PTEN* in significant rates, underlines the necessity to include gene panel testing in the clinical management of PC patients.

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P01.019.C Functional role of miR-93-5p in prostate cancer: from target gene identification to biomarker evaluation

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Background: Despite being one of the most prevalent malignancies among men, prostate cancer (PC) still lacks reliable non-invasive biomarkers for accurate diagnosis and patient stratification. Current methods, including prostate-specific antigen (PSA) testing, present specificity and prognostic value limitations. MicroRNAs have emerged as promising alternatives due to their stability in bodily fluids and regulatory role in gene expression. Among them, miR-93-5p has been linked to several malignancies, but its potential as a biomarker and functional relevance in PC remains unclear. This study explores its role in PC, focusing on its diagnostic and prognostic value.

Material and methods: miR-93-5p expression was analyzed in PC patients and control individuals using The Cancer Genome Atlas (n = 550) dataset, and an independent cohort of prostate tissue (n = 254) and plasma samples (n = 254). Using an integrative approach combining cell transfection assays, RNA sequencing, Western-Blot analysis, and computational affinity prediction studies, we identified miR-93-5p direct targets, which were further validated through in silico and in vitro analyses using the same cohorts.

Results: miR-93-5p was significantly upregulated in PC and its combination with PSA improved diagnostic accuracy over PSA alone. Functional studies revealed its involvement in key cancer-related pathways, including MAPK, FoxO, and TGF-beta. Target gene analysis identified *TGFBR2* and *SEMA7A* as direct targets, both showing significant downregulation in PC and a strong inverse correlation with miR-93-5p.

Conclusion: These findings support miR-93-5p as a promising biomarker for PC diagnosis and patient stratification, while also opening new perspectives for therapeutic strategies by revealing its key role in PC tumorigenesis.

C.O.I. Disclosure:

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P01.020.D Clinical syndromes linked to biallelic germline variants in MCM8 and MCM9

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Background: *MCM8* and *MCM9* are proposed cancer predisposition genes, linked to polyposis and early-onset cancer, in addition to hypogonadism. Given the uncertain range of phenotypic manifestations, this study aimed to delineate the molecular and clinical characteristics.

Material and methods: Collecting *MCM8/9* biallelics through case reports, ERN Genturis and analysing cancer risk with data from the 100K Genomes Project/UK biobank. Tumor analyses though WGS.

Results: We found significant enrichment of biallelic *MCM9* variants in individuals with colonic polyps (OR 6.51, 95%CI 1.24–34.11; $P = 0.03$), rectal polyps (OR 8.40, 95%CI 1.28–55.35; $P = 0.03$), and gastric cancer (OR 27.03, 95%CI 2.93–248.5; $P = 0.004$) in data from the 100K Genomes Project, compared to controls. No similar enrichment was found for *MCM8* or in the 200K UK Biobank. Likewise, in 26 *MCM8* and 28 *MCM9* variant carriers, we documented polyposis, gastric cancer, and early-onset colorectal cancer in *MCM9*, but not in *MCM8* carriers. Moreover, this case serie indicated that, beyond hypogonadism, biallelic *MCM8* and *MCM9* variants are associated with early-onset germ cell tumors (occurring <15 yr). Tumors from *MCM8*/*MCM9* variant carriers predominantly displayed clock-like mutational processes, with no evidence of DNA repair deficiency-associated signatures.

Conclusion: Collectively, our data indicates that biallelic *MCM9* variants are associated with polyposis, gastric cancer, and early-onset CRC, while both biallelic *MCM8* and *MCM9* variants are linked to hypogonadism and the early development of germ cell tumors. These findings underscore the importance of including *MCM8*/*MCM9* in diagnostic gene panels for certain clinical contexts and suggest that biallelic carriers may benefit from cancer surveillance.

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P01.021.A Integrative pathway analysis and epitope-based immunotherapy for targeting cancer stem cells in pancreatic cancer

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Background: Pancreatic cancer remains one of the deadliest malignancies due to late diagnosis, aggressive tumor biology, and therapy resistance. Cancer stem cells (CSCs) play a crucial role in tumor progression, metastasis, and immune evasion. Targeting CSCs through immunotherapy offers a promising strategy for improving patient outcomes.

Material and methods: We integrated bioinformatics and pathway analysis to identify key CSC markers (CD44, CD133, EPCAM) and their roles in pancreatic cancer. Epitope-based vaccine design was performed using immune-dominant peptides predicted for HLA-binding affinity. Identified epitopes were assessed for immunogenicity, safety, allergenicity, and population coverage. Pathway enrichment analysis was conducted to determine CSC-associated metabolic and signaling pathways.

Results: High-affinity HLA-A02:01 and HLA-A24:02-restricted epitopes were identified, demonstrating strong immunogenic potential. Pathway analysis revealed critical CSC survival mechanisms, including metabolic dysregulation and immune evasion pathways. Screening confirmed epitope safety and broad population applicability.

Conclusion: Our findings support the development of peptide-based vaccines targeting CSCs, providing a novel immunotherapeutic approach for pancreatic cancer. Future validation and clinical translation will be essential to enhancing treatment efficacy and overcoming therapy resistance.

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M. Darabi: None. **M. Zare:** None.

P01.022.B Distinct genomic and transcriptomic profiles of clear cell renal cell carcinomas and kidney cysts revealed in a von Hippel-Lindau disease patient

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Background: Patients with von Hippel-Lindau (VHL) disease develop multiple kidney cysts and clear cell renal cell carcinomas (ccRCCs) throughout their lifetime. While kidney cysts are typically considered precursors to ccRCCs, molecular evidence supporting this progression has been lacking.

Material and methods: To shed light on the molecular relationships and distinctions among these lesions, we analyzed a clinical case with multiple kidney cysts and tumors co-occurring at the time of resection. Comprehensive profiling was conducted using whole genome sequencing (WGS), RNA sequencing (RNA-seq), and pathological examinations on six ccRCCs and four cysts from the same VHL patient.

Results: Cysts and ccRCCs displayed distinct transcriptomic profiles: tumors exhibited increased glycolysis and hypoxia response, while cysts were enriched in extracellular matrix organization, inflammation, and markers of the nephron's distal portion. Deconvolution analysis revealed differing stromal and immune microenvironments, with cysts enriched in myofibroblast and plasma cell signatures, whereas tumors were associated with tumor-associated macrophages (TAMs) and tumor vasculature signatures. Genomic analysis showed that kidney cysts and ccRCCs were mostly clonally independent, with distinct somatic single-nucleotide variants' sets. While both lesions shared similar somatic mutational signatures, their somatic copy number landscapes diverged significantly. Notably, only tumors exhibited chromosome 3p loss, resulting in VHL loss of heterozygosity, without additional detectable driver mutations.

Conclusion: These findings reveal a molecular distinction between kidney cysts and ccRCCs, suggesting that these lesions can have distinct and independent origins, challenging the assumption of a linear cyst-to-tumor progression in VHL disease.

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P01.023.C EX^{2}TRICAN study: uncovering genetic predisposition factors in extreme cancer phenotypes through germline exome sequencing

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Background: Hereditary cancer syndromes often remain unexplained despite up-to-date panel-based genetic testing. Our study investigates the utility of germline exome sequencing (ES) to unravel cancer genetic predisposition by identifying new cancer genes in patients with extreme cancer phenotypes. Unexplained familial aggregation or early-onset cancers (before age 40, or before age 30 for breast cancer) were studied.

Methods: Germline ES was performed for all cases, in solo, duo, trio or quatuor strategy, depending on the family history and relatives' availability. Variants were prioritized using a dedicated algorithm, and a merged list of 1563 cancer genes from 3 cancer databases. Pathogenicity was assessed using ACMG/CanVIG-UK guidelines.

Results: Since 2017, 189 individuals from 7 different centers were included (Table 1). 51% (50/97) of affected cases harbored at least one genetic germline variant of interest. Several variants were prioritized for further study based on predicted loss-of-function, de novo status, or recurrence within the same pathology group (Table 2).

Discussion: This study demonstrates the added value of germline ES in providing insights into hereditary cancer, particularly by identifying novel candidate genes or questioning

the spectrum of known predisposition genes. Ongoing studies aim to refine these insights and confirm some of our candidates' involvement. Our findings support the broader use of germline ES in suspected hereditary cancer predisposition.

Table 1. Study population by cancer type and affected status.

Cancer type	Index cases	Affected relatives	Relatives with ambiguous status	Unaffected relatives	TOTAL
Breast	36	9	2	39	86
Ovarian	11	0	0	10	21
Colorectal	16	1	2	19	38
Other	20	4	0	20	44
TOTAL	83	14	4	88	189

Table 2. Main heterozygous germline variants of interest.

Cancer type	Gene	Variant	
Breast	*DNMT3A*	c.2411C>T	p.(Pro804Leu)
	ERCC4	c.2615A>G	p.(Asn872Ser)
	FANCA	c.3788_3790del	p.(Phe1263del)
Ovarian	*MSH3*	c.2814del	p.(Met939TrpfsTer18)
	CCBE1	c.683_684insT	p.(Leu229ProfsTer8)

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P01.024.D Germline pathogenic variants in *DOT1L* are associated with familial serrated polyposis syndrome

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Background: Serrated polyposis syndrome (SPS) is characterized by numerous and/or large serrated polyps and increased colorectal cancer risk. The underlying etiology is largely unknown(1). *DOT1L* (Disruptor of telomeric silencing 1-like), encodes for the only lysine methyltransferase that catalyzes the mono-, di- and trimethylation of histone H3 at lysine 79 (H3K79). This methylation is linked to active gene transcription and plays an important role in SPS (2). *DOT1L* has been proposed as a candidate gene for germline SPS predisposition (3). We aimed to provide functional evidence supporting *DOT1L* as a new hereditary gene for SPS.

Material and methods: Findings from a discovery cohort (39 patients, 16 SPS families), were replicated in 211 SPS patients. Two knock-in cellular models with the DOT1L rare, pathogenic genetic variants identified were generated with CRISPR-Cas9. We examined protein expression of di- and tri-H3K79 methylation, and target gene expression. An additional SPS cohort (184 patients) was checked for additional variants, where four potentially pathogenic variants were also identified in the additional SPS cohort.

Results: Two DOT1L variants (p.Asp1159His and p.Val1103Met) were functionally assessed by evaluating the global protein levels of H3K79me2 and me3, and expression of target genes. Cells showed an increase in global H3K79me2 coinciding with increased target gene expression of *MYC*, *CCND1* and *CDKN1A*, especially for p.Asp1159His.

Conclusion: Our findings suggest that *DOT1L* genetic variants can impact the global H3K79me2 pattern, therefore affecting the expression of target genes like *MYC*, *CCND1* and *CDKN1A*. This functional alteration might explain the mechanism underlying *DOT1L* SPS germline predisposition.

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P01.025.A Molecular and clinical differences between constitutional and somatic *POLE* and *POLD1* exonuclease domain pathogenic variants in cancer

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Background: Germline and somatic pathogenic variants in the exonuclease domain (ED) of *POLE* and *POLD1* impair the proofreading activity of the polymerases ε and δ, leading to hypermutated tumors. Despite shared mutational features, molecular and clinical differences between *POLE* and *POLD1* mutations, and between somatic and germline variants, remain incompletely understood.

Materials and methods: We systematically compared the molecular and clinical features of somatic and germline *POLE* and *POLD1* ED pathogenic variants (n = 31). The analysis considered associated clinical phenotypes, mismatch repair (MMR) deficiency status, tumor mutational burden (TMB), and mutational signatures. The study included 360 proofreading-deficient tumors from TCGA and COSMIC and 70 families with polymerase proofreading-associated polyposis.

Results: Somatic variants predominantly occurred in endometrial cancers, while colorectal cancer was most common in hereditary cases. Cancer risks and tumor spectra differed between *POLE* and *POLD1* mutation carriers. Aggressive clinical phenotypes were linked to specific germline *POLE* mutations or co-occurrence of germline pathogenic variants in *POLD1* or *POLE* ED and an MMR gene. Tumor mutational signatures analysis confirmed unique hypermutator profiles for *POLE* and *POLD1* proofreading deficiencies. In combination with MMR deficiency, tumors accumulated more mutations and had different mutational spectra. *POLE* deficiency showed haploinsufficiency, while *POLD1* required a somatic second hit or co-occurrence with MMR deficiency to trigger carcinogenesis.

Conclusions: Differences between *POLE* and *POLD1*, and between somatic and germline mutations influence clinical presentations, mutagenic potential, and reliance on cooperating defects in tumorigenesis. These insights advance understanding of proofreading-deficient cancers, with implications for diagnostics, genetic counseling, and precision oncology.

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P01.026.B New insights into variants in the *NF1* and *NF2* uORFs and their role in neurofibromatosis development

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Research Centre for Medical Genetics, Moscow, Russian Federation.

Background: Variants in uORFs cause hereditary diseases, primarily by creating an out-of-frame overlap with the downstream CDS, while other types of uORF variants remain unnoticed.

Material and methods: Variants altering *NF1*/*NF2* uORFs were identified using the uORF_annotator tool. Their impact on translation efficiency was experimentally assessed using a dual-luciferase assay and qPCR.

Results: Patients with neurofibromatosis, lacking variants in the coding sequences of the *NF1/NF2* genes, were analyzed for 5`UTR variants. Two novel variants in the *NF1* uORF were identified. We experimentally validated their effect on main CDS translation, along with several additional disease-associated *NF1/NF2* variants from HGMD and ClinVar. All tested variants altered uORF structure via frameshift or stop codon removal, causing an out-of-frame overlap with the main CDS, reducing protein translation, and contributing to disease. Next, we introduced additional SNVs that alter *NF1/NF2* uORFs structure without leading to overlap with the main CDS and measured their impact on protein translation efficiency. We found that variants altering the uORF length or the distance between its stop codon and the CDS start can significantly reduce the expression of the main CDS. In some cases, the level of this reduction is nearly identical to that caused by variants leading to out-of-frame overlaps. This suggests that such variants may also lead to the disease development, although they have not been previously described.

Conclusion: Using uORFs in the *NF1* and *NF2* genes as an example, we demonstrated that variants altering the uORF structure without affecting the main CDS can potentially cause inherited diseases.

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P01.028.D Optimising risk factor information in multifactorial risk assessment, including polygenic score, to inform risk-based breast cancerscreening

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Background: Risk-based breast cancer (BC) screening tailors age range, screening modalities and intervals to target women most likely to benefit. This study aims to identify the most important risk predictors for risk stratification, and to optimise data requirements for BC risk assessment in screening programmes.

Material and methods: We estimated 10-year BC risks using BOADICEA v.6 (CanRisk) in 3,753 women aged 40–70 with no prior cancer history, from the PERSPECTIVE I&I cohort. The risk classification ability of combinations of risk factors were evaluated against a full multifactorial model that included: questionnaire-based risk factors (QRFs), polygenic score (PGS), mammographic density (MD), and pedigree-structured multi-cancer family history up to second-degree relatives (FH).

Results: Under the full model 16.7% of women were at "higher-than-average" ($1.5 \leq RR < 2.7$) and 5.2% at "high" risk ($RR \geq 2.7$). Excluding PGS resulted in the overall highest reclassification rate (18.6%), followed by QRF (12.8%), MD (12.0%), and FH (10.3%). Including only first- and second-degree relatives' BC status yielded a comparable risk classification to the complete FH data (reclassification rate = 0.5%). Younger women (40–49) had higher reclassification rates when MD (17%) or FH (17%) were excluded, compared to women aged 50–70 (9.2% and 11.3%). Adding PGS to information already collected in breast screening programs in Canada brought risk classification close to the full model.

Conclusion: PGS contributes substantially to classification and could enhance risk-based BC screening recommendations. Younger women's risk predictions are particularly sensitive to the omission of FH and MD, emphasising the need to prioritise these factors in this demographic.

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P01.029.A The national landscape of cancer predisposition in Australia: an overview of carrier data submitted to the Inherited Cancer Connect (ICCon) database

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Background: The ICCon Database is a comprehensive national repository of genetic testing, demographic, and cancer data for carriers (≥18 years) of a pathogenic/likely pathogenic variant (P/LPV) in a cancer-predisposition gene. Here we review current carrier-data and describe the most frequently observed cancer-predisposition P/LPVs in Australia.

Material and methods: The ICCon Database currently houses data for 30,000+ carriers identified through one of Australia's 18 public Familial Cancer Centres between 1990s-2023 (67 unique genes; 70% female).

Results: In each Australian State, 50–59% of submitted carries have a P/LPV in *BRCA1/2*; the combined mismatch repair-genes are present in 16–27%. The combined *SDH*-genes are present in 2–4%, notably higher in Tasmania (10%). Other single-genes prominent nationally are *APC* (3–8%); *CHEK2* (1–3%); *PALB2* (1–3%); *FLCN* (1–2%). Other unique genes are present at similar frequencies (1–3%) in individual states.

The Ashkenazi Jewish BRCA1/2, and the European CHEK2, founder variants occur at the highest frequency nationally. Summary of the most frequently observed P/LPVs:

	Gene	Sequence Variation	% P/LPVs in same gene	% All P/LPVs
1	BRCA1	c.68_69del: p.Glu23Valfs*17	7	1.7
2	CHEK2	c.1100del: p.Thr367Metfs*15	71	1.6
3 =	BRCA1	c.5266dup: p.Gln1756Profs*74	6	1.5
4 =	BRCA2	c.5946del: p.Ser1982Argfs*22	5	1.5
5	BRCA2	c.6275_6276del: p.Leu2092Profs*7	4	1.3

The rate at which new carriers are detected in Australia more than doubled in 13 years, between 2010 and 2023.

Reported cancer diagnoses at the time of presentation largely align with known cancer-gene risk associations, but with an important minority of unexpected diagnoses.

Conclusion: Variations in gene and P/LPV frequency across states appear to reflect historical demographic differences. The data provides a detailed landscape of the evolution of cancer-predisposition in the clinical-setting and will be useful for planning future services and facilitating new cancer research.

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P01.030.B Germline pathogenic variants in ESMO-recommended genes found during molecular profiling of tumour tissues: results of the first 2 years of the FPG500 study

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Background: Comprehensive cancer genome profiling (CGP) is recommended for identifying actionable somatic mutations in select cancer patients, with somatic variants potentially linked to hereditary cancer syndromes (HCS).

Materials and methods: "FPG500" is a prospective trial enrolling cancer patients for somatic molecular assessment based on national guidelines. This analysis reports the prevalence of germline pathogenic variants (PVs) among patients enrolled from January 2022 to December 2023, profiled using TruSight Oncology 500 solution.

Results: Of 2696 cases with a CGP report, one fifth of cases with 623 suspected germline PVs were referred for genetic counseling, with a compliance to genetic counseling of 80%. 356 patients underwent germline testing and 75% harbored at least one PV, accounting for 10% of the entire cohort, with 68% of tested variants confirmed to be of germline origin. Interestingly, 24% of germline variants were found in an off-tumor context, while 17% of confirmed on-tumor germline PVs were in moderate-risk genes. Additional 19 PVs were identified using alternative panels due to either a suspicion raised by the results of other somatic tests or in patients referred to the genetics clinic based on clinical criteria.

Conclusions: This study confirms that approximately 10% of an unselected cancer population who underwent CGP is associated with an HCS. The somatic multigene panel allowed also the identification of a high percentage of germline variants as secondary findings or in mild- and low-penetrance genes. Integrating CGP with genetic counseling offers a comprehensive approach, aiding personalized treatment and broader familial risk assessment and preventive care.

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P01.031.C Solid tumors in Noonan syndrome, Costello syndrome, and related RASopathies: insights from a large monocentric cohort, updated genotype-phenotype analysis, and comprehensive literature review

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Background. The RAS-MAPK signaling cascade mediates cell response to various growth factors, cytokines and hormones, modulating cell proliferation, differentiation, and survival, which are key events implicated in tumorigenesis. RASopathies are a group of neurodevelopmental disorders with overlapping clinical presentations caused by enhanced signaling through the RAS-MAPK cascade(1). Among RASopathies, Noonan syndrome (NS), Costello syndrome (CS), and CBL-associated syndrome share an increased cancer risk, while data concerning other RASopathies remain anecdotal(2,3).

Methods: We assessed the prevalence and type of solid tumors in RASopathies by a retrospective analysis on a large monocentric cohort of affected individuals. We compared these findings with currently available public data collected through systematic literature review.

Results: This study presents the largest single-center cohort of individuals with RASopathies and solid tumors reported to date. It documents the occurrence of solid neoplasms in 10.8% of individuals with NS, 47.8% with CS, and 7.3% with cardio-facio-cutaneous syndrome (CFCS). Several CS patients experienced multiple tumors, while CFCS patients relapsing neoplasia. Our data confirms an earlier onset of solid tumors in RASopathies, especially in CS compared to the general population. Notably, *PTPN11*- and *SOS1*-positive NS patients exhibit a distinct spectrum of rare tumors, different from those observed in CS. Finally, we identify a subset of high-risk candidate variants in RASopathy genes that predispose to solid tumors.

Conclusion: The collected data provide further insights into RASopathies as cancer predisposition syndromes and emphasize the need for tailored surveillance and evidence-based clinical guidelines specific to each RASopathy subtype.

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P01.032.D Investigating the genetic predisposition of rare hereditary cancer syndromes via the Hellenic Precision Medicine Network

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Background: Greece lacks organized genetic centers, certified counselors and geneticists. Genetic testing for hereditary cancer is generally not reimbursed by national healthcare insurance policies, except in selected cases. As a result, patients and their families bear the financial burden. To address this gap, a state-funded program, Hellenic Network of Precision Medicine (HNPM), has been established to both investigate cancer biomarkers and hereditary cancer susceptibility. HNPM leverages the expertise of research and academic laboratories to seamlessly integrate diagnostic and research needs.

Material and methods: Through the dedicated HNPM portal, from September 2024 to January 2025, 192 patients (mean age at diagnosis 49.5 years; range 19–80 years) with malignant/pre-malignant diagnoses fulfilling international testing criteria were referred to the Human Molecular Genetics Laboratory, which serves as a reference laboratory in hereditary cancer in the last 30 years. Analysis was performed via massively parallel sequencing and MLPA.

Results: Overall, 20.8% (40/192) of patients carried germline pathogenic variants (PVs) distributed in six cancer susceptibility genes known to be associated with rare or ultra-rare cancer syndromes. The reported rate of variants of unknown significance (VUS) was 1%. A large fraction, i.e. 60%, of the families already proceeded to cascade testing for the familial PV.

Conclusion: This initial approach emphasizes the importance of an organized infrastructure, which includes laboratories with extensive expertise in the field. By offering *pro bono* genetic testing for hereditary cancer predisposition, a number of rare hereditary cancer cases are promptly identified whilst paving the way for personalized treatments and cancer prevention.

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P01.033.A Precision medicine in pediatric cancer patients: INT-per-Kids trial experience

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Background: Refractory/relapsing patients merit molecular tumor re-evaluation to inform about salvage targeted treatments. Precision medicine recently expanded this option to WES+RNA-seq tumor analyses. We present our initial experience, based on the first 41 pediatric patients enrolled in INT-per-Kids trial, focused to refractory/relapsing solid tumors.

Material and methods: Forty-one metastatic/relapsing tumor and matched normal samples were analyzed with KAPA DNA-HyperExome and KAPA RNA-HyperPrep next-generation-sequencing. Either formalin-fixed paraffin-embedded (FFPE) or frozen tumor tissue was used. Data were analyzed with Mercury bioinformatic platform (Integragen) and actionable alterations were verified by independent techniques. Therapeutic implications were discussed in multidisciplinary Molecular Tumor Board sessions. Ethical committee approval and informed consents were obtained.

Results: Technical success rate was 93% for DNA-analyses and 82% for RNA-analyses. Samples from FFPE provided significant inferior quality parameters. Pathology supervision revealed crucial for appropriate sample management. In 36/41 patients relevant, previously hidden information was revealed, including individual cases of high copy number amplification events, homozygous deletion events, rare fusion genes undetected at diagnosis stage, unsuspected very high tumor mutational burden. Target-based therapy was undertaken in 2 patients carrying actionable, "ready-to-use" alterations and additional 3/36 patients were enrolled in Phase 1 /2 trials based on investigational targets and drugs. No relevant/challenging incidental findings were reported.

Conclusion: We set up the laboratory steps for advanced molecular analyses in refractory/relapsing solid tumor pediatric patients. Results disclosed a number of impactful information and we anticipate that running these -omics approaches in earlier stages of disease in the frame of clinical trials will benefit patients' management.

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P01.034.B Identification of germline predisposition to colorectal cancer in a Spanish cohort of adolescent and young adult patients: DNA-RNA analysis results

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Background/objectives: Colorectal cancer (CRC) incidence has declined but early-onset cases are increasing globally (1). Our study focused on adolescents and young adults with CRC (AYACRC). The proportion of cases due to hereditary cancer is around 16–20% (2). We aimed to identify germline alterations predisposing to CRC.

Methods: Forty-three AYACRC patients (age range 19–45) formed the cohort, which included 27 women (62.8%). Only three patients presented mismatch-repair deficient (MMRd) tumors. Germline blood whole-genome sequencing (WGS) and RNA sequencing were performed and integrated. We scrutinized for rare (<1%), pathogenic variants, focusing on 1,657 genes reportedly involved in germline predisposition to cancer (3), or from CRC signaling pathways. When focusing on DNA-only results, we carefully inspected known hereditary cancer genes, especially those for CRC and breast cancer.

Results: Aberrant expression analysis did not detect any plausible hereditary cancer alteration. One splicing variation was linked with a *MSH2* variant (c.212-478T>G). Through DNA-only approach, we detected a variant in a CRC/breast cancer family (*ATM*, p.Ser917PhefsTer3), a controversial *MLH1* variant in a concordant MMRd case (p.K618A), *PMS1* alterations in 2 patients, suggestive variants in juvenile polyposis genes, and a pathogenic variant (p.Met1?) in *ARHGEF28*, a familial thyroid cancer candidate (4) in a CRC/thyroid cancer family.

Conclusion: Our approach for DNA-RNA analysis yield is limited. Germline predisposition could not be the main CRC susceptibility force for our AYA-CRC patients. Also, hereditary cancer pleiotropy could exist in some cases. This study is part of a multiomic effort and somatic, metabolomic or microbiome characterization may shed additional light.

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P01.036.D The Not-So Rare Tumour Risk Syndromes (RTRS): an European perspective on prevalence and healthcare

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Background: Rare Tumour Risk Syndromes (RTRS) are a subset of rare diseases, each defined as affecting fewer than 5:10.000 individuals. However, collectively, RTRS represent a significant clinical, social, and financial burden, requiring specialized diagnosis, continuous surveillance, and lifelong management. Herein, we aim at evaluating the prevalence of RTRS carriers receiving care in European Reference Centres.

Material and methods: Clinical data from eight RTRS (Hereditary Diffuse Gastric Cancer-HDGC, Gastrointestinal Stromal Tumour Syndrome-GIST, Birt-Hogg-Dubbé Syndrome- BHD, Hereditary Leiomyomatosis and Renal Cell Carcinomas- HLRCC, Li-Fraumeni Syndrome-LFS, PTEN Hamartoma Tumour Syndrome-PHTS, Familial Malignant Melanoma-FMM, and Peutz-Jeghers Syndrome-PJS) were collected from 2000 to 2024 across nine European Reference Centers in seven countries. Carrier families, variant carriers, and non-carriers were enrolled.

Results: A total of 1.108 RTRS families were identified (28% LFS, 26% PHTS, 16% PJS, 9% HDGC, 8% BHD, 6% HLRCC, 6% FMM, and 1% GIST). A total of 1.967 RTRS carriers were/are being offered multidisciplinary care to treat or prevent advanced disease, while 2.026 non-carriers were discharged, reducing unnecessary follow-ups and mental distress, and alleviating the burden on healthcare systems. Based on the total population of 16.6 million served by the nine centers, the prevalence of these RTRS carriers was calculated as 1.18:10,000.

Conclusion: LFS, PHTS, and PJS are the most prevalent RTRS in speacialized centers, collectively comprising 70% of families and carriers. While RTRS remain rare even when combined, their collective burden underscores the importance of European collaboration, specialized care, and tailored healthcare strategies to improve diagnosis, management, and policy decisions.

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P01.037.A ATM and hereditary cancer syndromes: a five-year retrospective analysis of oncogenetic testing in a french center

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Background: The tumor-suppressor gene *ATM*, encoding a serine/threonine kinase essential for DNA double-strand break repair, has emerged as a significant cancer susceptibility gene. Multiple case-control studies and large-scale genomic analyses have shown that heterozygous carriers of pathogenic (P) and likely pathogenic (LP) *ATM* variants are at increased cancer risk, particularly for breast and pancreatic malignancies. Recent data from the UK Biobank have further quantified these risks with robust statistical evidence. Despite accumulating molecular and epidemiological data, *ATM* is not currently included in French national guidelines for germline cancer predisposition testing.

Material and methods: We retrospectively analyzed (SeqONE™) negative NGS (Agilent™) data from 2500 individuals with hereditary breast-ovarian, digestive, or pancreatic cancer syndromes, referred for oncogenetic testing between 2019 and 2024 at the Laboratory of Solid Tumor Biology, CHU Montpellier. Data were re-screened with a specific focus on the *ATM* gene (not routinely included in standard genetic screening panels in France).

Results: Thirty-five patients (1.4%) carried heterozygous P or LP *ATM* variants, all loss-of-function. These variants were primarily associated with hereditary breast-ovarian cancer syndrome, characterized by a specific histological pattern, and to a lesser extent with digestive and pancreatic cancers. Compared with the European non-Finnish population in the gnomAD v3 non-cancer database (0.3%), the calculated relative risk (RR) was 3.9 across all cancer subtypes.

Conclusion: Given the relative frequency of *ATM* variants in our cohort and the elevated RR, the *ATM* gene should be incorporated into routine oncogenetic testing in France to enable an identification and a better management of at-risk individuals.

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P01.038.B Whole genome sequencing enhances the diagnostic yield in central nervous system tumours - technical advantages and limitations

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Background: Accurate diagnosis and prognostic stratification of central nervous system tumours remains a challenge due to their molecular complexity. We aimed to evaluate the added diagnostic

value of whole genome sequencing (WGS) compared to standard-of-care (SOC) molecular tests.

Material and methods: Fresh brain tumour samples from 255 patients (102 paediatric/TYA, 153 adult) were analysed by a multimodal NGS panel (covering 305 DNA and 76 RNA targets) and DNA methylation array (DKFZ). Comparative analysis with WGS data included detection of small nucleotide (SNVs) and structural variants (SVs), loss of heterozygosity and germline findings.

Results: WGS confirmed 96.7% of the known pathogenic SNVs and found an additional 90 novel variants. Germline variants were detected in 26 cases (identification rate of 10%). Additional findings to SOC included: four cases of high tumour mutational burden (TMB), chromoanagenesis events (33 cases), and genome-wide ploidy gains (40 cases). WGS did not tier 17 variants previously identified via SOC testing, including 14 SNVs and 3 SVs. Regular drop outs included TERT promoter variants which occur in a low complexity, high homopolymer region of the genome.

Conclusion: WGS offers a single-test molecular approach with improved diagnostic utility over the standard-of-care tests, currently with increased turnaround time. Challenges occur in regions of low complexity which can be compounded by poor mapping, low coverage, and high TMB. Further work must be undertaken to improve bioinformatic filtering, including white-listing, in order to ensure all relevant variants are tiered for analysis and prevent the need for manual interrogation of raw data.

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P01.039.C Contribution of *BRIP1* gene to cancer predisposition

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Background: BRIP1 plays a critical role in maintaining genome integrity through the Homologous Recombination pathway. Monoallelic germline pathogenic/likely pathogenic variants (gPV/gLPV) have been associated with an increased risk of cancers, notably breast/ovarian cancers. However, the comprehensive spectrum of cancers remains poorly characterized and carriers-specific recommendations lack precision.

Material and methods: We retrospectively analyzed *BRIP1* in patients tested for predisposition to breast/ovarian, prostate, digestive, pancreatic cancers and sarcomas at Institut Curie and Cochin Hospital (Paris-France). The analysis focused on identifying gPV/gLPV in *BRIP1*. Rare missense variants of uncertain significance (gnomAD frequency <0.01%) with a CADD score ≥20 (VUS^+) were also collected. Statistical enrichment analysis was performed using Fisher's exact test (v4.1.0 gnomAD cohort as a control population). Clinical and tumor data were collected.

Results: Between 2018 and 2021, 19,100 panels from 17,977 patients were analyzed, including 13,722 HBOC, 732 prostate, 3507 digestive, 998 pancreas and 141 sarcomas panels. A total of 201 variants was identified (1%) including 56 gPV/gLPV (0.3%) and 145 VUS^+ (0.8%). We identified 47 (0.3%), 1 (0.1%), 1 (0.1%), 5 (0.1%) and 2 (1.4%) gPV/gLPV in each group, respectively. The enrichment analysis suggested a significant association between gPV/gLPV and breast/ovarian cancers and sarcomas with odds ratio of 1.7 (IC[1.3–2.3]; $p = 0.001$) and 7 (IC[1.7–28.5]; $p = 0.003$), respectively.

Conclusion: Our results align with previous studies and suggest an expansion of the *BRIP1* tumor spectrum. Further analyses on paired tumor samples (biallelic inactivation and Homologous Recombination Deficiency) are planned to provide deeper insights into causality with potential therapeutic implications.

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P01.040.D Detecting circulating tumor DNA in archived whole blood samples from metastatic patients

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Background: The detection of circulating tumor DNA (ctDNA) through liquid biopsy has primarily focused on plasma from the blood of cancer patients. However, this method requires trained personnel, specific equipment, and several millilitres of blood for plasma collection and storage. We explored the possibility of detecting ctDNA directly in archived blood samples from cancer patients.

Material and methods: We retrospectively analysed matched whole blood ($n = 10$), and plasma ($n = 10$) collected more than ten years ago from melanoma patients. DNA was extracted from 9 tumours and sequenced at 30X whole exome sequencing to derive patient-specific mutations. We extracted DNA from blood and performed paired-end, shallow WGS. We analyzed the data for somatic copy number aberrations (SCNAs) tumour fraction estimation, and single nucleotide variants (SNVs).

Results: In archived blood, detected SCNAs reflected the profile observed in plasma and tumour from the same patients. Using the bioinformatic pipeline ichorCNA, tumour fraction ranged from 5% to 40% in blood. We identify distinct fragmentation profiles in

whole blood with nucleosome fragmentation patterns similar to plasma cfDNA. Analysis of SNVs by Integration of Variant Reads pipeline, detected ctDNA in nine out of the 10 patients with mutant allele fractions ranging from 1%–30%.

Conclusion: ctDNA can be detected directly from thawed whole blood and suggests the potential for ctDNA analysis in existing archived blood collections. Compared to conventional protocols based on plasma cfDNA, the proposed process is novel, simple and requires minimal blood volume, allowing existing biobanks to maximise discovery research across various types of cancer.

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P01.041.A SOSTAR: elucidating RNA isoform complexity across long read sequencing

Camille Aucouturier[1,2,3], *Laurent Castéra*[1,2], *Alexandre Atkinson*[1,2], *Thibaut Lavolé*[1,2], *Nicolas Goardon*[1,2], *Angélina Legros*[1], *Agathe Ricou*[1,2], *Flavie Boulouard*[1,2], *Dominique Vaur*[1,2], *Sophie Krieger*[1,2,3], *Raphaël Leman*[1,2]

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Background: Solving the structure of mRNA transcripts represents a significant challenge for both research and molecular diagnostic purposes. Current approaches lack a unified bioinformatic framework for accurate isoform annotation. To address it, we developed SOSTAR (iSofOrmS annoTAtoR), a versatile pipeline for assembly, quantification and annotation of RNA isoforms. This tool was validated using both short and long read sequencing data. Furthermore, the tool was extended to encompass the analysis of artificial constructions derived from multi-exonic minigene splicing assays.

Material and methods: From alignment and assembly achieved by minimap2 and StringTie tools respectively, SOSTAR enables comprehensive annotation which assigns an human-readable nomenclature to the isoforms. SOSTAR was validated on 40 samples including those with complex genetic alterations such as the insertion of an SVA element, genomic deletion and duplication, sequenced by both Illumina and Oxford Nanopore Technologies platforms. Twenty artificial constructions from 9 genes were considered to evaluate SOSTAR.

Results: The structure of transcripts was annotated with a resolution of one base relative to a reference transcript. All major alternative splicing events reported in the literature were identified and complex splicing events such as pseudoexons were accurately annotated. The application of SOSTAR pipeline allowed the comprehensive annotation of isoforms obtained from multi-exonic minigenes sequenced in a single experiment.

Conclusion: SOSTAR is a powerful tool for elucidating transcript structures from both short and long read sequencing data. It opens up new possibilities for the analysis of multi-functional splicing assays. To validate its diagnosis value, a cohort of 110 patients is under analysis.

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P01.042.B Whole exome sequencing identifies candidate genes for genetic predisposition to male breast cancer

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Background: In contrast to female breast cancer, male breast cancer (MBC) is a rare and still poorly understood malignancy but its incidence is increasing. Risk factors for MBC described to date include both environmental and genetic aetiologies, but the primary risk factor currently identified is the presence of a germline pathogenic variant or likely pathogenic variant in the *BRCA2, BRCA1* and/or *PALB2* genes.

Material and methods: To identify novel potential MBC-specific predisposition genes, we performed a large germline whole-exome sequencing (WES) study in 122 MBC patients without evidence of genetic predisposition due to *BRCA2, BRCA1* and/or *PALB2* genes, with and without family history of cancer. We then compared the frequency of occurrence of germline variants between the MBC cohort and a non-cancer male control population from GnomAD database. Only variants that were significantly more frequent in the MBC cohort (adjusted p-value < 0.05) were selected and then automatically classified to retain only pathogenic and likely pathogenic variants that could be associated with the risk of developing MBC.

Results: This work led us to identify, in the MBC population, germline variants in DNA repair pathways but also in other cellular functions, suggesting that these pathways may be involved in MBC risk.

Conclusion: To our knowledge, this work is the first one performing a whole exome study in a large MBC population. Considering the role of genetics and environment, an additional analysis is under current to identify a multifactorial profile of MBC risk by combining patient's genetic and environmental data.

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P01.043.C *ELOC*, a novel predisposition gene responsible for the von Hippel-Lindau disease

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Background: Von Hippel-Lindau disease (VHL) is a rare genetic disease characterized by predisposition to hemangioblastomas of the central nervous system (CNS-HB) and retina (R-HB), clear cell renal cell carcinomas (ccRCC), pheochromocytomas (PCC) and pancreatic neuroendocrine tumours (PNET). VHL is caused by germline heterozygous pathogenic variants (PV) in the *VHL* tumor-suppressor gene. Here, we report the exploration of two typical VHL families without any identified *VHL* PV (*VHL*wt).

Material: Family A: proband presented R-HB at 29yo, multiple CNS-HB at 34yo and bilateral ccRCC at 37yo. At 57yo, his mother developed bilateral ccRCC, bilateral R-Hb and PCC. Family B: 38yo female patient with bilateral ccRCC and probable PNET at 42yo.

Results: No *VHL* variant was found in both families. In family A, a germline *ELOC* p.(Glu92Gly) variant was identified in both affected patients. In patient B, the p.(Tyr79Cys) *ELOC* PV was found in germline DNA. In all analyzed tumor tissues (n = 7), the *ELOC* variant was associated with LOH.

Conclusion: *ELOC* gene encodes the elongin C protein, which binds to pVHL. *ELOC* PVs have been already described as somatic driver mutations in sporadic ccRCCs but litterature reports only one case of patient with germline p.(Tyr79Cys) *ELOC* variant (Andreou *et al*., 2022). Here we report another case with clinical diagnostic for VHL carrying the same germline VP and also a family presenting a new *ELOC* variant segregating with the disease. Therefore, we propose *ELOC* as a novel VHL predisposition gene, emphasizing the need for European collaboration to refine the clinical spectrum of ELOC-associated VHL disease.

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P01.044.D *DICER1* in pediatric and adult cancer predisposition populations: prevalence, phenotypes and mosaics

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	ID	Sex	HGVS genomic GRCh37	HGVS transcript	HGVS predicted protein change	VAF (%)	ClinGen's criteria	ClinGen"s classification	Additional germline findings	Reason for the study (age)	Other tumors (age)
Pediatric cohort	**P1**	F	g.95583017G > A	c.1525C > T	p.(Arg509Ter)	44.44	PVS1, PS2, PS4, PM2_supporting, PP4	Pathogenic	-	Follicular adenoma of the thyroid (2)	Thyroid FND (4)
	P2	F	g.95572492del	c.2873del	p.(Ser958Ilefs*23)	43.33	PVS1, PM2_supporting	Likely pathogenic	-	Sertoli-Leydig cell tumor (10)	Thyroid FND (10)
	P3	F	g.95570193G > T	c.3540C > A	p.(Tyr1180Ter)	45.85	PVS1, PS2, PS4, PM2_supporting, PP4	Pathogenic	-	Pleuropulmonary blastoma (2)	-
Adult cohort	**A1**	F	g.95577652dup	c.2256+3dup	p.?	47.22	PM2_supporting	VUS	*MSH6* PV	Poorly differentiated endometrioid endometrial adenocarcinoma (78)	-
	A2	F	g.95570461dup	c.3272dup	p.(Tyr1091Ter)	41.43	PVS1, PM2_supporting	Likely pathogenic	-	Clear cell carcinoma of the ovary (55)	Thyroid cancer (30)
	A3	F	g.95562700_95562702del	c.4560_4562del	p.(Glu1520del)	43.43	PM2_supporting, PM4_supporting	VUS	-	Basal Cell Carcinoma (56) Melanoma (57, 57)	Tubular adenoma (52) Trichilemmal cyst (56) Mature ganglioneuroma (58) Thyroid FND (58) Melanoma (59) Clear cell acanthoma (59)
	A4	F	g.95560463T > C	c.5126A > G	p.(Asp1709Gly)	20.85	PS2, PS3_supporting, PS4_supporting, PM1, PM2_supporting, PP3	Pathogenic	*BRCA1* PV	HGSC of the ovary (53)	Diffuse thyroid hyperplasia (50)
	A5	F	g.95556862del	c.5742del	p.(Asn1915Ilefs*13)	50.80	PVS1_moderate, PM2_supporting	VUS	-	Mucinous ovarian adenocarcinoma (57)	-

Background: DICER1 tumor predisposition syndrome (DTPS) is a rare pleiotropic hereditary condition affecting children and young adults. Identification of *DICER1* carriers is key for prevention and actionability in families. However, DTPS diagnosis is hindered by its incomplete penetrance and broad phenotypic spectrum. We aimed to characterize *DICER1* variants in large cancer predisposition cohorts to determine their prevalence in the actual clinical setting and elucidate the clinical extent of the disease.

Methods: We analyzed *DICER1* sequencing data from 92 children and 6108 adults with a suspected cancer predisposition syndrome. Clinical and *DICER1* somatic data from selected carriers and public datasets were studied. Functional analyses were conducted on a variant of unknown significance.

Results: The prevalence of germline *DICER1* PVs was 1:30 in children and 1:3054 in adults (Table 1). No adult referral phenotype was a known DTPS-associated tumor, although 3/5 carriers also developed thyroid alterations. We provide functional evidence supporting the pathogenicity of a novel in-frame deletion. A 56-year-old woman with an ovarian carcinoma and a toxic diffuse thyroid hyperplasia was found to have a postzygotic hotspot missense.

Conclusion: The prevalence of *DICER1* PVs in cancer predisposition populations was 5–6 times that reported in the general population. Pediatric-onset DTPS is well characterized, whereas adult carriers mainly present with thyroid abnormalities in the absence of DICER1-related family history, thus requiring accurate criteria for its identification when in constellation with other tumor types. Postzygotic hotspot missenses may exist without the expected severe phenotype.

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P01.045.A Impact of updated variant classification criteria on *BRCA1* and *BRCA2* gene variants: a retrospective analysis

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Background: The ClinGen/ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) *BRCA1* and *BRCA2* Expert Panel (VCEP) recently updated the 2015 ACMG/AMP variant interpretation guidelines, refining criteria for pathogenicity and benignity and introducing new classification combinations (PMID: 39142283).

Material and methods: To assess the impact of these revised guidelines, the clinical lab Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Ipatimup Diagnostics) performed a reclassification of all previously identified *BRCA1* and *BRCA2* germline variants. Variants were categorized by type and reassessed based on a comprehensive review of evidence, including population databases, literature, in silico prediction models, and laboratory-developed tools.

Results: A total of 154 *BRCA1* and 322 *BRCA2* variants were reviewed. While molecular diagnoses remained largely unchanged, there was a notable reduction in VUS and an increase in LB/B classifications. This shift was largely due to the activation of the 'BP1_Strong' criteria, which allowed the reclassification of certain synonymous and missense variants as LB, provided they do not affect critical functional domains or splicing. Reclassified variants with clinical implications prompted updated reports for clinicians.

Conclusion: Implementing the new ClinGen ENIGMA VCEP criteria for *BRCA1* and *BRCA2* has reduced inconclusive results in genetic testing by almost 70%. This advancement enhances diagnostic accuracy and supports more reliable patient management, ultimately improving the quality of care.

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P01.046.B DNA long-read sequencing, an interest for genetics predispositions to breast and ovarian cancer

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Background: Current molecular diagnostic methods for Hereditary Breast and Ovarian Cancer (HBOC) are based on short-read sequencing (SRS) of exons and flanking regions. These approaches can fail to detect complex events. Emergence of long-read sequencing (LRS) enables identification of these events, no matter how complex. We propose here a complete LRS method: from DNA extraction to final bioinformatic analysis.

Material and methods: We selected 200 families whose phenotype was in favour of HBOC syndrome and SRS results were negative. After extraction of high molecular weight DNA, sequencing was performed on the PromethION P2 solo using adaptive sampling technology on a panel of 161 genes. After demultiplexing and basecalling with Dorado, the pipeline performs alignment with minimap2, single nucleotide variants calling by Clair3 and annotation with SnpEff, VEP and GreenVaran. Structural variants were called by Sniffles and annotated by AnnotSV and GreenVaran. Copy number variants were investigated using CNVkit.

Results: Two hundred patients were sequenced. The average coverage for our panel genes was 50 reads. Adaptive sampling gave an average coverage of 5 reads for the other regions, allowing for shallow sequencing. All positive controls were detected by the pipeline. Although the analyses are being processed, we already fully characterized 3 insertions of Alu elements, 2 duplications.

Conclusion: In conclusion, LRS makes allows the characterisation of complex events. Our approach facilitates the analysis of a large number of patients by LRS. The analysis of the 200 patients could explain part of the missing hereditary through the discovery of new variants.

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P01.047.C Comprehensive systematic literature review of *BRCA1* and *BRCA2* variants uncovers the significance of ENIGMA guidelines on clinically actionable variants

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Background: *BRCA1* and *BRCA2* mutations significantly increase the risk of developing breast, ovarian, and other cancers. Rigorous classification is crucial for diagnosis and patient management.

Material and methods: Mastermind Genomic Search Engine (Genomenon) was used to collect BRCA1/2 variants from the literature, followed by a semi-automated curation with manual expert review according to the ACMG/AMP criteria. The classification of variants, including the application of PM5(S), was completed according to the ClinGen ENIGMA *BRCA1* and *BRCA2* Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for BRCA1 Version 1.1.0. The PM5(S) was applied for variants with premature termination codon (PTC) in an exon with an existing pathogenic PTC variant.

Results: Systematic literature review of 16,223 articles revealed 25,046 *BRCA1* and 11,799 *BRCA2* variants, respectively. The curation of *BRCA1* resulted in 8146 Pathogenic/Likely Pathogenic, 13,979 VUS, and 2,908 Benign/Likely Benign variants. The curation of *BRCA2* resulted in 4153 Pathogenic/Likely Pathogenic, 7050 VUS, and 592 Benign/Likely Benign variants. PM5(S) was applied to 1,242 *BRCA1* variants and 19 *BRCA2* variants which led to the upgrade of 1,077 BRCA1/2 variants. Out of those, 500 variants were upgraded from VUS to pathogenic and 577 BRCA1/2 variants were upgraded from LP to P. Furthermore, 225 BRCA1/2 that were upgraded from VUS to pathogenic did not have patient data.

Conclusion: The application of the PM5(S) criterion led to the clinically significant upgrade of BRCA1/2 variants. This recalibration of variant classifications, driven by the nuanced interpretation of PM5(S), resulted in significant shifts in the overall assessment framework.

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P01.048.D Consequences of *BCL2* promoter/5'UTR mutations in lymphoid malignancies

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Background: B-cell non-Hodgkin lymphomas (BNHL) and chronic lymphocytic leukemia (CLL) frequently exhibit dysregulation of the anti-apoptotic *BCL2* gene family. This study investigates the association between mutations in the *BCL2* promoter and 5'UTR regions and the gene expression levels, alongside their broader impact on molecular pathways in tumor-derived samples.

Material and methods: Somatic variants from WGS and transcriptomic data from RNA-seq were obtained from the PCAWG (106 BNHL and 73 CLL samples) and the Hartwig repository (25 BNHL samples). Differential expression analysis was conducted using limma, and pathway-level effects were examined through Gene Set Enrichment Analysis (GSEA).

Results: 40% of BNHL and 16% of CLL samples were mutated in the *BCL2* promoter/5'UTR regions. The mutated samples exhibited a ~5-fold and ~1.2-fold higher expression of *BCL2* in BNHL and CLL respectively, though the causal relationship remains uncertain. Notably, basal *BCL2* expression in CLL was approximately 10 times higher than in BNHL. Differential expression analysis revealed 955 upregulated and 990 downregulated genes in mutated samples. GSEA highlighted significant downregulation of the cell cycle and DNA replication genes, with concurrent upregulation of cell adhesion molecules pathway. Dysregulated genes included *IGF2BP1*, *TERT*, and *SOX11*. Modest downregulation of *APOBEC3A* and *AIDCA* was also observed.

Conclusion: *BCL2* 5'UTR/promoter mutations are associated with increased expression levels and alterations in key oncogenic and immune-related genes/pathways. Although a direct causal link cannot be confirmed, these findings highlight the potential significance of these mutations in BNHL and CLL pathogenesis. Further studies are essential to clarify their mechanistic role and therapeutic/diagnostic potential.

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P01.049.A Tumor exome sequencing highly contributes to elucidating extreme cancer phenotypes: complementary results of the EX²TRICAN study

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Table. TES results in 10 tumors showing necessary and sufficient somatic drivers, providing sporadic cancer diagnosis.

Tumor	MSS/ MSI status	GIS score	TMB score	SBS signatures (if relevant)	Drivers CNV	Drivers SNV				
						Gene	Nucleotide variation	Protein variation	VAF (%)	LOH
Colorectal adenocarcinoma	MSS	21	**196**	**SBS10: 0,9 (*POLE/ POLD1*)**	None	***POLE***	**c.857C > G**	**p.(Pro286Arg)**	**27,9**	**LOH**
Colorectal adenocarcinoma	MSS	6	**37**	**SBS10: 0,2 (*POLE/ POLD1*)**	None	***POLD1***	**c.953A > G**	**p.(Glu318Gly)**	**12**	-
Colorectal mucinous adenocarcinoma	MSS	39	4,3	SBS6: 0,16 (dMMR)	None	***APC***	**c.3842C > A**	**p.(Ser1281Ter)**	**41,2**	**LOH**
						KRAS	**c.35G > T**	**p.(Gly12Val)**	**15,3**	-
Low grade serous ovarian carcinoma	MSS	10	3,1	SBS6: 0,2 (dMMR)	None	*MSH3*	c.2814del	p.(Met939TrpfsTer18)	50 (G)	-
						KRAS	**c.35G > A**	**p.(Gly12Asp)**	**50,7**	-
Mucinous ovarian carcinoma	MSS	25	3,6	SBS24: 0,21 (Aflatoxin)	None	***KRAS***	**c.35G > T**	**p.(Gly12Val)**	**20,6**	-
Low grade serous ovarian carcinoma	MSS	11	4,5	SBS43: 0,15 (Unknown)	None	***KRAS***	**c.35G > A**	**p.(Gly12Asp)**	**22,2**	-
Low grade serous ovarian carcinoma	MSS	2	2,4	None	None	***BRAF***	**c.1799T > A**	**p.(Val600Glu)**	**43,3**	-
Astrocytoma	MSI-L	25	43	SBS11: 0,57 (Temozolomide) and SBS15: 0,19 (dMMR)	None	***ATRX***	**c.2202_2208del**	**p.(Met734IlefsTer6)**	**60,5**	-
						IDH1	**c.395G > A**	**p.(Arg132His)**	**40,6**	-
Cutaneous squamous cell carcinoma	MSI-L	39	7,6	None	None	***CDKN2A***	**c.247C > T**	**p.(His83Tyr)**	**65**	**LOH**
Rectosigmoid low grade tubular adenoma	MSS	2	3,3	SBS6: 0,18 (dMMR)	19qter complete loss (no obvious TSG in the deleted region)	***APC***	**c.4057_4070del**	**p.(Glu1353SerfsTer17)**	**53,8**	**LOH**

All variations were found somatic excepted the MSH3 c.2814del that was found germline (G).

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Background: Tumor Exome Sequencing (TES) is a powerful tool to identify strong molecular drivers and key genomic biomarkers in most cancer types, like GIS, TMB or mutational signatures. In the context of EX[2]TRICAN study, based on Germline Exome Sequencing (GES) in extreme cancer phenotypes, we evaluated the contribution of TES in the elucidation of molecular, possibly alternative causes of these phenotypes.

Material and methods: TES+GES was performed in 26 tumors among 97 cases from EX[2]TRICAN study. SNV, CNV variants and various genomic signatures were obtained (Table).

Results: 26 tumors from 15 tumor types were sequenced. 3 groups were identified: sporadic tumors with necessary and sufficient somatic drivers (n = 10, 38%, Table), tumors with somatic drivers that do not confirm sporadicity (n = 6, 23%), tumors without any somatic driver (n = 10, 38%). Surprisingly, we could identify sporadic colorectal cancer caused by POLE or POLD1 somatic mutations in 2 young patients aged 31 and 35.

Conclusion: TES is a powerful tool to identify causes of extreme cancer phenotypes where genetic predisposition is suspected: in 37% of cases, the presence of causal somatic drivers provides arguments for the diagnosis of sporadic cancer. TES showed its utility in confirming the causality of a candidate variant (either germline or somatic), when associated with specific signatures like TMB, SBS, GIS. This approach also provides useful information for family screening and follow-up decisions. These findings encourage us to integrate TES in the diagnosis strategy of extreme cancer phenotypes.

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P01.050.B Re-evaluation of *ATM* variants reported as variants of uncertain significance in breast cancer patients: impact of updated ClinGen guidelines

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Background: The *ATM* gene plays a critical role in DNA damage response, and its germline variants have been associated with hereditary breast, pancreatic, prostate and gastric cancers. Because of reduced penetrance and limited knowledge of functional effects, pathogenicity assessment is complicated, which in turn results in variants of uncertain significance (VUS). The 2024 ClinGen Hereditary Breast-Ovarian and Pancreatic Variant Curation Expert Panel (HBOP-VCEP) guidelines provided gene-specific modifications to the interpretation framework, aiming to better delineate classification of *ATM* variants.

Material and methods: In this study, a total of 465 patients who were referred to our center and underwent next-generation sequencing panel for breast cancer between 2020 and 2024 were retrospectively examined. All *ATM* variants that are reported as VUS were included 47 unique VUS was detected in 67 patients. Reanalysis of these variants were performed in accordance with updated ClinGen HBOP-VCEP specifications.

Results: Nineteen out of 47 variants (40.4%) were reclassified: 16 as likely benign, 2 as benign, and 1 as likely pathogenic. The refined classification was primarily driven by the incorporation of updated population frequency thresholds, updated in silico score thresholds, and updated segregation data evidence strengths in the new criteria. One variant was reclassified, not because of guideline specifications, but because of recent emergence of functional evidence.

Conclusion: This study highlights the impact of periodic re-evaluation of VUS in clinically significant genes like ATM. The implementation of updated, gene-specific guidelines enhances the accuracy of interpretation, reduces the uncertainty in genetic counseling, and improves clinical decision-making for breast cancer patients.

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P01.051.C Genotype and phenotype in carriers of pathogenic variants in *RAD51C* and *RAD51D* genes

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Background: Beyond *BRCA1/2*, other genes were recently implicated in Hereditary Breast/Ovarian Cancer, including *RAD51C* and *RAD51D*, for which evidence on cancer risks needs to be collected.

Material and methods: The records of families carrying variants in *RAD51C/D*, either diagnosed by our Laboratory or referred to our Cancer Genetics Clinic after testing, were reviewed.

Results: *RAD51C/D* variants were identified in 24 individuals from 11 families (table 1).

Gene	Variant	N. of families	N. of carriers (patients/ asymptomatic relatives)
RAD51C	c.1026+5_1026+7delGTA	2	5 (4/1)
RAD51C	c.905-2_905-1del	3	5 (4/1)
RAD51C	c.904+5G>T	2	3 (2/1)
RAD51D	c.694C > T (p.Arg232Ter)	2	6 (2/4)
RAD51D	c.803G > A (p.Trp268Ter)	1	3 (3/0)
RAD51D	c.817C > T (p.Arg273Ter)	1	2 (1/1)

Seven carriers (29,2%) developed Ovarian Cancer (OC), 7 (29,2%) Breast Cancer (BC), one (4,2%) both, one had colon cancer (4,2%), while 8 (33,3%), were asymptomatic relatives (6 women, 2 men, aged 34–73). Four cancer patients (16,7%) developed additional malignancies: cholangiocarcinoma, melanoma, rectal

adenocarcinoma, and leukemia. OC was diagnosed at an average age of 57 years (range 50–76) and had high histologic grade in all cases: 6 serous type, one endometrioid, one undifferentiated. After a mean time from diagnosis of 140 months (range: 13–259), 6 patients were alive, 2 deceased (mean time from diagnosis to death: 133 month). BC was diagnosed at an average age of 55 years (range 34–68). Triple-negative histology was observed in 5 cases (62.5%); two patients (29%) developed metachronous contralateral triple-negative carcinomas. After a mean time from diagnosis of 56 months (range: 2–211), all patients were alive.

Conclusions: Data observed suggest favorable prognosis for *RAD51C/D*-associated OC, prevalence of triple-negative histology for BC and tendency to develop multiple primary cancers. If confirmed, these features will inform tailored carrier management.

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P01.052.D Exploring cfDNA copy number profiles to monitor tumor dynamics in pancreatic adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) remains a leading cause of cancer-related mortality due to its aggressive nature and late-stage diagnosis. Circulating tumor DNA (ctDNA) offers a minimally invasive approach to monitor tumor dynamics. We investigated cfDNA profiles across PDAC stages and treatment timelines, utilizing shallow whole genome sequencing (sWGS).

Material and methods: We collected 121 cfDNA samples from 59 PDAC patients. The cfDNA samples were analyzed with sWGS, using WISECONDOR for copy number profile abnormality (CPA) scores and ichorCNA for tumor fractions (TF). We evaluated whether these markers could distinguish PDAC stages and monitor disease progression, comparing their utility to CA19-9 levels and imaging.

Results: Patients with resectable tumors had significantly lower CPA scores (0.65 ± 0.35) and TF ($3.6 \pm 1.9\%$) than metastatic patients (CPA: 3.47 ± 1.7, TF: $15.1 \pm 8.0\%$). Borderline resectable, locally advanced and early metastatic cases had CPA (0.61 ± 0.21) and TF ($3.3 \pm 1.1\%$) values similar to resectable cases, indicating that CPA and TF are primarily increased in metastatic PDAC. CPA and TF were significantly correlated, though preliminary data suggests CPA may be more robust. No significant correlation was found between cfDNA concentration and CPA or TF. Both markers showed no clear correlation with CA19-9 but, in some patients, aligned better with disease state than CA19-9.

Conclusions: sWGS of cfDNA provides a cost-effective tool that may complement CA19-9 and imaging, particularly in metastasized PDAC. Ongoing analyses include investigating whether alterations of specific genomic regions are associated with prognosis.

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P01.053.A Molecular implications of transposable element activity in non-muscle invasive bladder cancer progression

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Background: Non-muscle invasive bladder cancer (NMIBC) exhibits great molecular heterogeneity. Understanding the genetic and epigenetic alterations underlying NMIBC subtypes is crucial for prognosis and therapeutic advancements. Recent studies have suggested a role for transposable elements (TEs) in oncogenic processes, but their impact on NMIBC remains largely unexplored.

Material and methods: Tumor samples from 18 NMIBC patients were analyzed using whole-exome sequencing (WES) to identify key mutations, revealing frequent alterations in *KMT2C, KDM6A, EP300, FGFR3*, and *KMT2D*. Molecular subtypes using the consensusNMIBC classifier were determined via RNA sequencing (RNA-seq). Differential gene expression analysis was conducted between Class 1 ($n = 9$), associated with favorable prognosis, and Class 2a ($n = 8$), characterized by poor prognosis. TE activity was assessed using the SQuIRE tool, and transcription factor (TF) motif enrichment analysis was performed using Homer.

Results: TE activity was significantly elevated in Class 2a tumors, particularly in LTR, DNA, LINE, and SINE elements. Locus-specific TE activation analysis identified 247 genes associated with TE expression, predominantly linked to cell cycle regulation. Spearman correlation analysis for expression levels of TE and genes within the same topologically associating domains (TADs) identified 428 genes. Homer motif analysis revealed significant enrichment of PAX6 binding sites on genomic sequences of TEs, and PAX6 expression was notably upregulated in Class 2a tumors, validated in an independent dataset.

Conclusion: These findings suggest a potential role of TE activation in driving oncogenic transcriptional programs in aggressive NMIBC subtypes. The association between TE activity and PAX6 upregulation highlights a novel regulatory mechanism in NMIBC progression.

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P01.055.C Effect of BAP1 inactivation on uveal melanoma class-specific epigenome formation in an isogenic BAP1 knockout model

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Background: Uveal melanoma (UM) is the most common primary ocular malignancy in adults. The two main classes of UM associated with metastatic progression can be distinguished by molecular features, including chromosome 3 status or DNA methylation patterns. The poor prognosis monosomy 3 UM class is characterised by inactivating mutations of the TSG BAP1, which encodes an enzyme that deubiquitinates histone H2A at lysine 119. To test the impact of BAP1 on the formation of the UM class-specific DNA methylation pattern, we generated an isogenic *BAP1* knockout (KO) cell culture model.

Material and methods: The UM 92-1 cell line was subjected to CRISPR-Cas9-mediated genome editing targeting the *BAP1* gene. Whole genome methylation analysis was performed by EM (enzymatic modification) sequencing with a median coverage of 15-fold.

Results: We generated 4 different *BAP1* KO cell lines with. Western blotting confirmed the absence of BAP1 and increased H2A mono-ubiquitination in all *BAP1* KO clones. Principal component analysis of genome-wide methylation data comparing *BAP1* WT and *BAP1* KO cells grouped samples according to their *BAP1* genotype and showed a consistent *BAP1*-dependent methylation pattern. There was no obvious difference in the methylation pattern between early and late passages after genome editing, suggesting that methylation changes are stably transferred over many passages.

Conclusion: *BAP1* inactivation in UM cell line with disomy 3 results in a stable change of the genome wide methylation pattern. However, to achieve the full M3 epi-phenotype additional factors are needed.

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P01.056.D Unraveling the role of ROS1 in breast cCancer: functional impact of the c.6005+2T > G variant

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Background: We recently discovered novel damaging variants in hereditary breast cancer cases in ROS1, a receptor tyrosine kinase with unknown ligands and functions, including the heterozygous variant c.6005+2T > G. This variant disrupts a donor splice site, resulting in the use of a cryptic splice site with partial intron retention and insertion of a premature stop codon in ROS1.

Material and methods: We utilized skin-derived fibroblasts from two first-degree cousins with early-onset BC carrying the c.6005+2T > G variant and control cells to evaluate tumor-related properties, perform RNA sequencing (RNAseq) analysis, and study mitochondrial alterations.

Results: Western blot analysis in mutant fibroblasts vs. controls indicated that the c.6005+2T > G variant was translated into a shorter protein due to the use of a premature stop codon. Compared to controls, mutant fibroblasts showed an altered ROS1 localization at the plasma membrane via immunofluorescence labeling, coupled to an increased migration rate via wound healing assay. RNAseq identified 2186 differentially expressed genes with a P-value < 10-3 between mutant and control fibroblasts, with significant dysregulation of genes related to migration and extracellular matrix organization, confirmed by real-time quantitative PCR. Furthermore, RNAseq analysis showed a significant dysregulation of mitochondrial genes, such as the mitochondrial iron-sulphur clusters, downregulated in mutant fibroblasts. Tetramethylrhodamine staining showed a mitochondrial hyperfusion in mutant fibroblasts, supporting mitochondrial alterations. Electron microscopy analyses are currently ongoing to define in further detail the subcellular organization of ROS1-mutant cells.

Conclusion: The findings suggest that mutant ROS1 enhances migration capacity and modifies mitochondrial functions, supporting a role of ROS1 in BC development.

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P01.057.A Mainstreaming the diagnosis of lynch syndrome (LS) in colorectal cancer (CRC) patients: the ItaLynch study

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Background: LS is highly underdiagnosed worldwide. The ItaLynch study aims to evaluate an oncologist-led diagnostic pathway for LS integrating germline genetic testing (GGT) into oncology.

Material and methods: ItaLynch is a prospective Italian study on Mismatch deficient CRC patients. It started in 2021 and is ongoing in 23 centers. The diagnostic pathway is based on four key steps: Universal Screening (dMMR testing by immunohistochemistry- IHC); Reflex Testing in MLH1-deficient (dMLH1) patients ($BRAF^{V600E}$ and, if wild-type, MLH1 promoter-methylation); Lynch Alert (LA, positive or negative) added to all dMMR CRC pathology reports, based on patients' likelihood of LS; Oncologist-Led Mainstream GGT for patients with a positive LA. Carriers of a germline pathogenic variant (PV), and those with VUS or clinical red flags and non-informative test results, are referred to genetic counseling (GC).

Results: As of Dec. 19/2024, 1146 dMMR CRC patients have been enrolled. Among the 937 patients eligible, 714 (76%) were dMLH1, and 223 (24%) displayed loss of non-MLH1 protein (68%). At the time of writing, out of 321 LA-positive patients (34%), 217 have undergone oncologist-led GGT (68%): 97 carried an LS-associated PV (45%), 14 a VUS (6%). Post-test GC was attended by 70/97 PV-carriers (72%) and 12/14 patients with VUS (86%).

Conclusion: The LA flagging system that identifies dMMR CRC patients with a high likelihood of LS, together with oncologist-led GGT, obtained a high diagnostic yield in our large cohort of dMMR CRCs. Further analyses are ongoing to evaluate the performance of and explore barriers and facilitators in the ItaLynch pathway

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P01.058.B Decoding the puzzle of human somatic mtDNA mutagenesis: bridging species life-history traits and organ-specific cellular properties

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Background: Mitochondrial DNA (mtDNA) mutagenesis in human cancers is still enigmatic: external mutagens like UV light or tobacco smoke, known to impact nuclear DNA, don't seem to affect mtDNA. This discrepancy suggests a dominant, mitochondria-specific internal mutagen. To understand this, we analyzed mtDNA mutational spectra across a wide range of vertebrates, uncovering that some mutations (A > G on the heavy chain) are linked to internal, age-related chemical damage, likely oxidative (https://doi.org/10.1093/nar/gkac779). Others (C > T on the heavy chain) stem from internal replication processes (https://doi.org/10.1101/2023.12.08.570826). Leveraging our insights, we hypothesized that in somatic mtDNA mutagenesis, slow-dividing cells would exhibit more damage-related mutations, while fast-dividing cells would show a predominance of replication-associated mutations, i.e. we posited a variable damage-to-replication ratio (A > G/C > T) across tissues with different replication rates.

Material and methods: To test this, we reanalyzed the two most comprehensive human mtDNA datasets: the TCGA, with 7611 mutations across 37 cancer types and 21 tissues, and the GTEx, with 2762 mutations in 25 healthy tissues.

Results: In both datasets, a higher damage-to-replication ratio was found in organs with slow-dividing cells (neurons, ovary, heart, skeletal muscles) and a lower ratio in those with rapid cell division (colon, intestine, stomach, vagina). Furthermore, a decrease in this ratio in late-stage somatic mutations across all cancer types (TCGA data) aligns with the anticipated increase in cell turnover during carcinogenesis.

Conclusion: Our research shows that mtDNA mutations can reveal cell traits, unveiling their potential importance in biomedical applications.

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P01.060.D The role of CREBBP in refining stratification of pediatric patients with B-cell acute lymphoblastic leukemia

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Background: Around half of the relapses in pediatric B-cell acute lymphoblastic leukemia (B-ALL) occur in patients initially classified as standard-risk. There is a growing need for refining the stratification in this group of patients.

Material and methods: A total of 55 pediatric patients with B-ALL were included in this study. All patients were treated according to the ALL-IC-BFM 2002 protocol and five of them experienced disease relapse in a median follow-up of 48 months. Numerical/structural chromosomal abnormalities and copy number alterations in disease-related genes were analyzed using RT-PCR and MLPA methods. Whole exome sequencing was performed for all patients with relapse (samples obtained both at diagnosis and at relapse) and for five additional patients in remission.

Results: Alterations in the CREBBP gene were identified in 4/5 (80%) of the patients with relapse, two of whom exhibited high-hyperdiploidy and standard-risk features. The mutations were persistent in both diagnostic and relapse clones in one patient (c.3836+1G > A), acquired at relapse in two patients (p.R1446H; p.H1487Y), or involved a change in the same amino acid in one patient (R1446H at diagnosis; R1446C at relapse). None of the examined patients in remission featured alterations in this gene. The remaining patient with relapse had TP53 and EBF1 deletions, which were rare or absent in our cohort.

Conclusion: Screening for alterations in CREBBP gene at diagnosis and/or during chemotherapy could contribute to the identification of a significant number of patients with predefined or acquired chemoresistant clones, which may improve risk stratification in pediatric B-ALL.

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P01.061.A Cancer diagnoses in patients with neurofibromatosis type 1 - a registry-based study

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Background: Neurofibromatosis type 1 (NF1) is a multisystem disorder caused by pathogenic variants (PV) in the *NF1* gene and characterised by distinctive cutaneous, ocular, neurological, cardiovascular, and musculoskeletal features as well as an increased cancer risk. Our analysis aimed to estimate the prevalence of non-neurofibroma neoplasms in a group of adult NF1 patients referred for tumour surveillance.

Material and methods: This study included all NF1 patients referred to the Department of Clinical Cancer Genetics, Institute of Oncology Ljubljana, between 2019 and 2024. Their medical records were reviewed, and the diagnoses of cancer/neoplasia were verified using data from the Slovenian Cancer Registry.

Results: We identified 73 patients (median age 42, range 18–75) with a clinical diagnosis of NF1 which was confirmed using genetic testing in 95.9% of all cases. The PV were presumed to be de novo in 52% of our patients. Most patients were female (71.2%) and had no diagnosis of cancer (57.5%). Of the 42.4% who developed a non-neurofibroma neoplasm, 30.1% had one diagnosis and 12.3% had multiple. Sarcomas were the most common neoplasm, seen in 15%. Of these, 8.2% had GIST, 4.1% MPNST, and 2.7% other sarcomas. Breast cancer was diagnosed in 11% of NF1 patients, all of them female. Gliomas were reported in 8.2% and pheochromocytomas in 5.5%. Interestingly, 2 patients (2.7%) were diagnosed with polycythaemia vera.

Conclusion: The prevalence of neoplasia in our NF1 patients was high but comparable to other studies, with an excess of cancers known to be associated with the disorder.

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P01.062.B Spatial transcriptomics reveal differentially expressed genes in pancreatic neuroendocrine tumors

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Background: Pancreatic neuroendocrine tumors (PanNETs) arise from α and β cells of islets of Langerhans. These neoplasms make up about 7% of all neuroendocrine tumors and less than 2% of all pancreatic neoplasms. Because PanNETs are uncommon, studies thoroughly examining their overall transcriptomic landscape are scarce.

Material and methods: We employed GeoMx digital spatial profiling (DSP) in this study to characterise expression in eight PanNETs. Gene expression from 45 regions of interest from tumor and and 18 from normal islet tissue were used for DSP. These specific tissue compartments were identified using morphology markers.

Results: Total of 360 unique differentially expressed genes (DEG) (FDR < 0.05 and Log2FC > 0.5) were detected when comparing different PanNET grades with normal islet tissues. Majority of these were downregulated in tumor tissue. Twenty-six of all DEG were overlapping across all three tumor grades and five genes (RBP4, SPINK1, PLA2G1B, EGR1, and CD99) were consistently downregulated with relatively high Log2FC below −1.27. EGR1 and CD99 are of particular interest in PanNETs. EGR1 is known to activate p53/TP53 and TGFB1 expression, potentially preventing tumor formation. Our data show inverse correlation of EGR1 and higher tumor grade. Meanwhile, CD99 has been linked to markers of poorer PanNET prognosis, including local invasion, lymph node metastasis, and lymphatic or blood vessel invasion.

Conclusion: Using spatial transcriptomics, we have identified multiple DEG between PanNET and normal islet cells of which at least two are likely to be tumor development related and are consistently downregulated across all tumor grades.

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P01.063.C Demonstration of a splice site effect of the missense p.(Glu224Ala) variant in TP53 in a Li-Fraumeni family

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Background: Carriers of pathogenic *TP53* germline variants have a higher risk of developing rhabdomyosarcoma. Here we report the case of a two-years-old child, deceased for a retroperitoneal rhabdomyosarcoma, carrying the maternally inherited c.671A>C p.(Glu224Ala) germline variant in the *TP53* gene, considered of uncertain significance (VUS). Functional assays were performed to verify the hypothesis of a possible role of the variant.

Material and methods: The variant was identified by whole exome sequencing analysis on the family trio. In vitro experiments on yeast, along with in silico analyses- minigene assay and cDNA analysis from maternally derived cells- were applied.

Results: Assays in yeast and mammalian cells demonstrated that the c.671A>C p.(Glu224Ala) missense variant has a wild-type functional profile. The variant affects the last codon of the exon 6 at junction with the intron, and SpliceAI predicted the alteration of the acceptor splicing with significant delta value effect. A minigene approach demonstrated the skipping of exon 6, with a frameshift in the coding sequence and the generation of a premature stop codon, that may likely target the variant transcript to the nonsense mediated mRNA decay. The loss of heterozygosity in *TP53* mRNA in maternally derived cells supported this hypothesis.

Conclusions: We demonstrated that the c.671A > C substitution alters P53 function by impairing the splicing rather than by altering the protein structure through the aminoacidic change. We collected functional data suitable to support the re-classification of a VUS into likely pathogenic and provided a conclusive molecular diagnosis useful for the proper family management.

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P01.064.D Longitudinal measurement of mutated *EGFR* ctDNA in plasma of advanced NSCLC patients on first-line tyrosine kinase inhibitors - a potential for early detection of drug resistance and therapy effectiveness monitoring

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Background: Non-invasive access to circulating tumor DNA (ctDNA) in plasma of non-small cell lung cancer (NSCLC) patients enables the dynamic monitoring of mutated *EGFR* gene (*mtEGFR*) levels during treatment with EGFR tyrosine kinase inhibitors (TKIs) instead of retrospective evaluation of re-biopsied tumor tissue. We evaluated the clinical value of longitudinal measurement of *mtEGFR* levels for the real-time treatment monitoring and early detection of drug resistance in advanced NSCLC patients receiving first-line EGFR-TKIs.

Material and methods: Peripheral blood was prospectively collected from 52 advanced NSCLC patients at the time of diagnosis (baseline) and then every month during the EGFR-TKI treatment until clinical progression. The *mtEGFR* was analyzed semiquantitively using cobas EGFR Mutation Test v2 (Roche, Germany) and verified using ddPCR.

Results: The median (min-max) progression-free survival (PFS) was 10.3 (1.8–35.6) months. Significant differences in PFS were found between patients who demonstrated complete clearance of baseline *mtEGFR* level in plasma within 1–2 month of treatment and patients whose *mtEGFR* levels were similar or higher than baseline [12 (4.5–35.6) vs. 8.35 (1.8–17.5) months, $p = 0.0099$], and patients with undetectable *mtEGFR* in plasma at diagnosis and during treatment [9.8 (1.8–30) months, $p = 0.04620$]. A drug-resistance variant, p.T790M, was detected in plasma of 13/52 (25%) patients before or upon clinical progression. Cobas test demonstrated high concordance with ddPCR ($R = 0.76$, $p < 0.0001$).

Conclusion: The dynamic changes of plasma *mtEGFR* levels in NSCLC patients reflect well their responses to EGFR-TKIs. The longitudinal monitoring of plasma *mtEGFR* levels allows to observe disease progression on EGFR-TKIs much earlier than conventional imaging techniques.

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P01.065.A *MUTYH*-associated polyposis: genotype-phenotype correlations in a single-centre cohort of 170 patients

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Background: Biallelic germline pathogenic variants (PVs) in MUTYH are responsible of MUTYH-Associated Polyposis (MAP), an autosomal recessive disorder associated with polyposis and increased risk of colorectal cancer (CRC).

Material and methods: Retrospective review of clinical and genotype data of patients with confirmed genetic diagnosis of MAP included in the Hereditary Colorectal Cancer Registry of Fondazione IRCCS Istituto Nazionale dei Tumori (Milan). Clinical information considered were: age at polyposis diagnosis, number of polyps, occurrence and age at CRC diagnosis, occurrence of multifocal and/or metachronous CRC, upper gastrointestinal (UGI) adenomas and extracolonic tumors.

Results: 170 MAP patients, belonging to 131 families, were included in the study. Eighteen different *MUTYH* pathogenic/likely pathogenic variants were identified. The most frequent were p.(Tyr179Cys), p.(Gly396Asp) and p.(Glu480del) (NM_001128425.2), with allele frequencies of 33%, 23% and 12%, respectively. Patients homozygotes for p.(Gly396Asp) or compound heterozygotes for p.(Gly396Asp) and a second PV ($n = 56$) showed a significantly later age at diagnosis compared to patients carrying other *MUTYH* PVs ($n = 114$) both for polyposis (51.7 vs 43.6 years) and CRC (54.6 vs 44.8 years). Furthermore, patients carrying at least one p.(Gly396Asp) variant also had a lower incidence of UGI adenomas (2.4% vs 23.3%).

Conclusion: The present study provides further evidences of genotype-phenotype correlations in MAP. The variant p.(Gly396Asp) is associated with lower UGI adenoma risk and a later polyposis and CRC onset, in accordance with previous reports and functional assays. Given the rarity of MAP, multicenter studies are necessary to explore the possibility of personalizing surveillance programs according to genotype.

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P01.066.B Comparative analysis of circular RNA and messenger RNA profiles in microsatellite stable and unstable colorectal cancers

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Background: Circular RNAs (circRNAs), emerging regulators of genetic information, share the spliceosome biogenesis pathway with messenger RNAs (mRNAs) and influence their expression. Evidence increasingly supports their role in colorectal cancer (CRC) tumorigenesis. This study explores circRNAs in two distinct

pathways of colorectal tumorigenesis: microsatellite instability (MSI) and microsatellite stability (MSS).

Material and methods: Circular (circ) and linear (lin) exon-exon junctions were quantified using exon-specific probes targeting 48 involved in CRC predisposition and tumorigenesis process. RNA was extracted from colorectal FFPE samples (stage 1 to 4 adenocarcinoma and adenomas). MSS-IHC tumors (MSS-TT; n = 21) and adjacent normal tissue (MSS-NT; n = 16) were selected from patients with a severe personal or familial cancer history without a known genetic cause. MSI-IHC tumors (MSI-TT; n = 13) and normal adjacent tissue (MSI-NT; n = 14) were also analyzed.

Results: Principal component analysis distinguished NT from TT samples based on circular and linear junction sums. CircRNA abundance was higher in samples with elevated muscle content (Kruskal-Wallis test p-value = 0.034). Linear regression, adjusted for muscle content, showed significantly reduced global circRNA levels in tumors compared to healthy tissues (MSI and MSS combined, p-value = 0.00268). In the MSS group, significant differences were observed between MSS-NT and MSS-TT for circ/lin ratios, and for linear and circular junction counts for specific genes. MSI analysis revealed distinct gene profiles, with significant differences only in linear junction counts.

Conclusion: Our study suggests differences in circRNA profiles between MSI and MSS tissues. This potential pathway-specific regulation might be linked to splicing mechanism alterations involving circRNA synthesis.

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P01.067.C Pre-test group genetic counselling for patients seeking cancer susceptibility predictive testing - an innovative service delivery initiative

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Background: Point of care testing and personalised medicine have begun to change management for patients in the cancer pathway while generating familial risk information for their at-risk relatives. Constraints in NHS funding, workforce availability and digital infrastructures have led to family members waiting for prolonged periods to access clinical genetic services thereby delaying predictive testing. Group genetic counselling for patients at risk of high-risk cancer susceptibility familial pathogenic variants was piloted at the Guy's regional genetics service to expand capacity and reduce waiting times.

Methods: Suitable patients were given information describing the model with consent forms for participation. The group genetic counselling ran for an hour with a focus on information provision to facilitate decision making. Each individual patient was then seen separately by a clinician for a twenty-minute genetic counselling consultation. We ran this both virtually and in person for families with hereditary breast ovarian cancer and Lynch syndrome.

Results: We delivered 17 group sessions of 180 patients with 96 % accepting the offer. Likert scales were used to capture patient experience and feedback before and after the clinic. A 20% increase in capacity was enabled within the service. Patients and staff reported a positive experience with this model.

Conclusion: Group genetic counselling has the potential to transform care for patients without compromising quality of care. Patients reported utility in the shorter individual sessions which allowed personalised decision making. This novel model of care can be adopted across health services to enable shorter waiting times.

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P01.068.D Liquid- and tissue-biopsy based genotyping for efficacy determination of anti-EGFR re-challenge in *RAS* wild-type advanced colorectal cancer (A-REPEAT study)

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Background: Despite therapeutic advancements, metastatic colorectal cancer (mCRC) remains a leading cause of death, with a 5-year survival rate of 14%. Treatment for *RAS* wild-type mCRC patients includes chemotherapy and an anti-epidermal growth factor receptor (anti-EGFR) agent as first-line therapy, followed by second-line treatment with chemotherapy plus an anti-vascular endothelial growth factor (anti-VEGF) agent. However, patients eventually progress with limited therapeutic options for successive lines. The A-REPEAT study (NCT03311750) evaluated the efficacy of anti-EGFR re-challenge as third-line therapy.

Materials and methods: Twenty-three *RAS* wild-type mCRC patients were recruited upon progression to second-line treatment. The primary objective was to evaluate the response rate (RR) to third-line treatment with panitumumab plus chemotherapy. Blood was serially obtained at diagnosis and every 2 months and subjected to the NeoThetis Colorectal cancer liquid biopsy assay (Medicover Genetics) while tissue samples were tested using a custom Ampliseq assay, to identify factors predictive of response to anti-EGFR re-challenge.

Results: The objective RR was 13% while disease control rate was 52%. NGS genotyping of tissue and liquid biopsies revealed *TP53*, *APC*, *KRAS* and *NRAS* as the most frequently altered genes. About half of the patients tested via liquid biopsy had *RAS* mutations prior to anti-EGFR re-challenge. Patient response or control of disease did not correlate with the plasma *RAS* status, possibly due to the small cohort size.

Conclusion: Liquid biopsies have the potential to guide anti-EGFR re-challenge in mCRC patients. Larger cohorts are required to determine the role of *RAS* and other biomarkers in response to anti-EGFR re-challenge.

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P01.069.A Impact of rare synonymous variants on cancer predisposition

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Background: Synonymous variants (sSNVs), despite not altering protein sequences, can influence gene regulation, including splicing. Unlike missense variants (mSNVs), the role of sSNVs in cancer predisposition remains poorly understood. This study investigates the functional impact of sSNVs in neuroblastoma, a pediatric cancer arising from aberrant neural crest development.

Material and methods: Whole-exome sequencing was conducted on 724 neuroblastoma cases and 2858 healthy controls. Rare variants (minor allele frequency [MAF] < 1%) were analyzed in 138 cancer predisposition genes (CPGs) using pathogenicity prediction tools, including synVEP, REVEL, PhyloP, FastCons, GERP, and SpliceAI.

Results: Considering all variants, we found similar rates of rare sSNVs and mSNVs in cases (16.9% and 27.3%, respectively) and in controls (18.7% and 32.6%, respectively). Moreover, 38.2% of rare sSNVs were predicted to be pathogenic by synVEP (scores ≥ 0.75), compared to 4.3% of rare mSNVs by REVEL (scores ≥ 0.75). Rare sSNVs were identified in 102 (74%) of the 138 CPGs in patients. Rare sSNVs in CPGs demonstrated significantly higher conservation compared to non-CPGs (PhyloP, $p = 0.02$; FastCons, $p = 0.009$; GERP, $p = 0.03$). Additionally, 21% of these variants recurred in at least two patients, a pattern also observed for mSNVs. Splicing analysis predicted disruptions in key genes, including SMARCA4, PALB2, FANCA and ATM (SpliceAI > 0.5), already found mutated by mSNVs in neuroblastoma patients.

Conclusion: Our findings suggest that sSNVs share pathogenic features with mSNVs, suggesting their functional impact. This highlights the importance of integrating sSNVs into cancer predisposition analyses.

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P01.070.B Integrating somatic mutation testing in Estonia: widening treatment options for ovarian cancer

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Background: In Estonia, ~140 cases of ovarian cancer (OC) are diagnosed each year. Somatic testing was incorporated into the routine care of OC patients in 2020. This study aimed to investigate the landscape of actionable variants (AV) in the tumor tissues of Estonian OC patients to identify patients who can benefit from targeted therapy.

Material and methods: The study included 346 OC tumors referred from tertiary hospitals to the Genetics and Personalized Medicine Clinic at Tartu University Hospital between 2020 and 2024 for comprehensive tumor profiling. Tumor samples were analyzed using the Illumina TruSight Oncology 500 somatic mutation gene panel (523 genes). Homologous recombination deficiency (HRD) testing was implemented in 2023.

Results: The uptake of somatic testing increased from 39 samples in 2020 to 115 samples in 2024. AV were determined according to OncoKB level 1/2 in solid tumors. In our study, 28.9% (91/346) had actionable single nucleotide variants. The primary AV were found in *BRCA1* 14.2% (49/346) and *BRCA2* 5.2% (18/346). Additionally, eight samples (2.3%) had actionable amplifications, seven in *ERBB2* and one in *MET* gene. Furthermore, four samples had actionable fusions, two in *FGFR2* gene. A high tumor mutation burden was observed in 4% (14/346), and microsatellite instability was found in 2.9% (10/346). HRD was identified in 36.2% (55/152) of samples.

Conclusion: The diagnostic efficacy of OncoKB level 1/2 actionable gene alterations in OC samples in Estonia was 29.8% (103/346). These patients can potentially be offered targeted therapy or included in clinical trials.

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P01.071.C Phenotypic spectrum in 170 patients with *MUTYH*-associated polyposis: results from a hereditary colorectal cancer registry

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Background: *MUTYH*-associated Polyposis (MAP) predisposes to adenomas and colorectal cancer (CRC). However, the phenotypic manifestations are variable and controversial.

Material and methods: Retrospective review of clinical information of patients with genetically confirmed MAP included in the Hereditary Colorectal Cancer Registry at the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan.

Results: Overall, 170 MAP patients, belonging to 131 families, were identified: the mean age at polyposis diagnosis was 46.5 years (range 21–75), and the majority (110/170; 64.7%) presented symptomatically. Total polyp count was provided for 101 patients

(59.4%), and 31.7% (32/101) reported more than 100 polyps. Sixty-seven individuals (39.4%) had hyperplastic and/or serrated lesions in addition to adenomas. More than half of the patients (101/170; 59.4%) developed CRC; of these, thirty-five (34.6%) had multifocal tumors and thirteen metachronous tumors (12.9%). The mean age at first CRC diagnosis was 48.1 years (range 21–75) and most patients (94/170; 55.3%) presented both polyps and CRC at diagnosis. Overall, 148 patients (87%) underwent at least one surgical treatment and in 66.2% (98/148) of these cases a total colectomy was performed. Among the 127 patients (74.7%) who underwent upper gastrointestinal tract (UGI) endoscopy, twenty-one (16.5%) presented duodenal adenomas. Extra-colonic malignancies were diagnosed in forty-one patients (24.1%), with endometrium (Standardized Incidence Ratio: 7.2), thyroid (SIR: 9.9), lower urinary tract (SIR: 6.4) and skin tumors (SIR: 6.9) having a higher incidence than the general population.

Conclusion: A comprehensive characterization of the phenotypic variability in MAP patients is essential to improve diagnosis and management.

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P01.072.D Comprehensive genomic and transcriptomic analysis enables molecularly-guided therapy options in advanced mesothelioma

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Background/objectives: Mesothelioma is a rare and aggressive disease with poor prognosis. Molecularly guided therapy is currently not part of standard care. We characterized a cohort of patients with advanced mesotheliomas clinically and molecularly using a broad multiomics approach.

Methods: 51 mesothelioma cases, including 21 peritoneal, 28 pleural, one synchronous and one pericardial mesothelioma, from the prospective observational German multicenter precision oncology program DKFZ/NCT/DKTK MASTER were included. Retrospective data analysis of 44 cases included parallel tumor and control genome or exome sequencing and transcriptome analysis. A molecular tumor board (MTB) assessed clinical actionability of somatic and germline alterations.

Results: 37/44 (84%) samples undergoing retrospective analysis demonstrated positivity in at least one biomarker indicating deficiency of homologous recombination (HR). Germline variant evaluation across 141 cancer predisposition genes (CPGs) revealed 13 pathogenic germline variants (PGVs) in 13/51 (25%) patients, ten thereof in genes associated with HR and eight in autosomal dominant CPGs. PGVs in *BAP1* occurred thrice. Loss of heterozygosity was observed in four tumors with PGVs in autosomal dominant CPGs (*ATM*, *BAP1*, *BRCA2*, *NF1*). PGVs impacted therapy recommendations (TR) in two patients, targeting impaired DNA damage response. Among 46/51 (90%) patients with TR, tyrosine kinase inhibition was recommended most frequently. TR were applied in six cases; three patients received immune checkpoint inhibitors.

Conclusion: Comprehensive molecular analysis provides insight into therapeutic potential. Based on the PGV yield and the potential clinical impact on patients and their relatives, parallel germline testing and molecular tumor characterization should be considered for all mesothelioma patients. **Grants:** DKFZ-HIPO-H021

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P01.073.A Uncovering novel structural variants in pediatric low grade gliomas

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Background: Despite the high success rate of treatment in pediatric patients with low grade gliomas (LGGs), some children have LGGs that behave more aggressively, leading to multiple recurrences, metastasis, and/or death. The reason for this discrepancy is not fully understood but may be due to the underlying differences in genomic composition. Short-read sequencing presents gaps in structural variant (SV) identification due to its underlying methodology, which creates a gap in our understanding of variants that influence tumor behavior and treatment response.

Material and methods: Here, we used optical genome mapping (OGM) to detect SV in a cohort of aggressive (postmortem, metastatic, or recurrent following standard of care therapy) and non-aggressive (surgery alone) LGG samples.

Results: The initial analysis focused on identification of known clinically significant fusions, such as the KIAA1549::BRAF fusion. OGM successfully identified these fusions in LGG samples with varying levels of variant allele fraction (VAF), supporting that LGG patients have different degrees of cell compositions carrying SVs. Further stratification of samples based on aggressive vs non-aggressive tumor behavior showed that aggressive tumors on average contain more SVs, including large chromosomal aberrations, than their non-aggressive counterparts.

Conclusion: This preliminary data demonstrates that the application of OGM in metastatic, refractory, and recurrent pediatric LGGs to define SV patterns could elucidate novel mechanisms involved in tumor behavior.

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P01.075.C Development of a risk score based on clinical-pathological features to predict the presence of germline*BRCA1/2*pathogenic variants in ovarian cancer patients

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Background: As the identification of germline *BRCA1/2* pathogenic variants is crucial to inform ovarian cancer (OC) treatment, here we aimed to develop a model to predict *BRCA1/2* status in OC patients.

Material and methods: The association between clinical-pathological features and *BRCA1/2* status was analyzed in a series of 1009 OC patients, using Fisher's exact test. Logistic regression models and a Decision Tree Classification algorithm were then used to develop a risk score.

Results: Compared to non-carriers, *BRCA1/2* carriers (216; 21.4%) presented significantly ($p < 0.05$) more frequently with serous histotype non-low-grade (92.3% vs. 71.6%), family OC history (31.6% vs. 5.7%), family breast cancer (BC) history (53.7% vs. 31.6%), previous BC (20.9% vs. 8.5%), advanced stage (78.8% vs. 69.5%) and younger age (56.9 vs. 60.8 years). Multivariable logistic regression confirmed histotype, family history of BC/OC, previous BC and age as independently and significantly associated with *BRCA1/2* status. ORs derived after calibration according to Decision Tree Classification algorithm results were used to assign weights from 1 to 3 to each feature (serous histotype, non-low-grade = 3, low-grade serous histotype/high-grade endometrioid histotype/family OC history = 2, age at diagnosis<50 years/family and personal BC history = 1) and to develop a score ranging from 0 to 10, associated with increasing risk of *BRCA1/2* variants (from 0.6% for score 0 to 88% for score ≥ 7). The AUC of the score was 0.78 (95% CI 0.74–0.82) and the optimal cut-off was ≥4 points (sensitivity 81%, specificity 62.3%).

Conclusion: The proposed risk score may support clinicians in the assessment of OC patients.

C.O.I. Disclosure:

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P01.076.D Distinct de novo *NF1* variants in gametes of affected parents and multiple complex events underline the high mutation rate in Type I Neurofibromatosis

Enrico Ambrosini[1], *Davide Martorana*[1], *Fiorenza Soli*[2], *Antonietta Taiani*[3], *Ilenia Rita Cannizzaro*[3], *Sabrina Busciglio*[3], *Elisa Gravagno*[1], *Sofia Cesarini*[3], *Patrizia Caggiati*[1], *Vera Uliana*[1], *Valeria Barili*[3], *Antonio Percesepe*[1,3]

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Background: The NF1 gene shows one of the highest mutation rates, estimated to be on the order of 10^{-4} per generation, with 50% of NF1 patients harboring de novo variants. Due to this high frequency of new mutational events, mostly on the paternal allele, co-occurrence of two or more different *NF1* pathogenic variants has been reported in at least 11 independent families.

Material and methods: We employed next-generation sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA) and long-read sequencing to investigate four families exhibiting peculiar mutational events related to the *NF1* high mutation rate.

Results: In two unrelated families, the proband displayed a de novo NF1 variant, while their respective affected father carried a different pathogenic variant. In both cases, the mothers showed no signs and tested negative. Long-read approach revealed that the de novo event took place on the paternal allele. Another NF1 patient, with a severe pseudoarthrosis of the tibia, showed three alternative nucleotides (G > T/C) at position 2376 of *NF1*, with variant fractions of 41%, 37% and 22%, respectively. Sanger sequencing confirmed the triple peak, suggesting the occurrence of two independent mutational events in a mosaic state. Finally, in a fourth patient we identified a deletion encompassing *NF1* and other 5′-contiguous genes, alongside a duplication at the adjacent 3′-end (involving *UTP6*, *SUZ12* and *LRRC37B*), suggesting a complex rearrangement.

Conclusion: The *NF1* high mutation rate should be considered in the diagnostic process and may complicate genetic counselling, altering recurrence risk and potentially explaining localized clinical manifestations or negative NGS results.

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P01.078.B Germline genomic testing in pediatric oncology - insights from a single-center experience

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Background: Up to 8–12% of childhood cancers are attributed to germline mutations in cancer-predisposing genes (CPGs). Israel's unique population - characterized by founder mutations and a high consanguinity rate - may influence the prevalence of germline CPG variants in pediatric cancer. However, data on Israeli pediatric patients remain scarce. This study evaluates the diagnostic yield of germline genomic testing in pediatric oncology patients at a newly established genetics clinic.

Material and methods: We retrospectively analyzed pediatric oncology patients (≤25 years) referred to the Genetics Institute at Tel Aviv Sourasky Medical Center (September 2019-December 2024). Genetic testing (panel/whole-exome sequencing) was recommended following clinical evaluation on an individualized basis. We determined the overall diagnostic yield, then stratified results by demographics, tumor type, and syndromic features.

Results: Among 147 referred patients (93 male, 54 female; ages 3 months-24 years, mean 8 years), 92 (63%) underwent genomic testing. Pathogenic/likely pathogenic variants were identified in 18/92 (20%) across multiple CPGs. Among mutation-positive patients, 56% (10/18) had additional systemic findings, compared to 18% (13/74) of mutation-negative patients ($p = 0.0019$). The highest CPG detection rates were in soft tissue sarcomas (3/7, 43%), Wilms tumor (1/3, 33%), neuroblastoma (2/7, 29%), leukemia (3/14, 21%), and CNS tumors (5/25, 20%).

Conclusion: Our findings suggest a higher-than-expected diagnostic yield of germline genomic testing in Israeli pediatric oncology patients, potentially influenced by population-specific factors. The association between pathogenic variants and syndromic features underscores the importance of comprehensive clinical evaluation. These results support tailored genetic counseling and expanded genomic testing in pediatric oncology.

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P01.079.C Molecular profiling of VHL mutations in sporadic ccRCC patients from India

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Background: Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cancer, with von Hippel-Lindau (VHL) gene alterations playing a key role in its pathogenesis. While global studies have extensively reported on VHL mutations, there is limited data from Indian subcontinent. This study aimed to characterize VHL gene variants in sporadic ccRCC patients from this region and explore their clinical significance.

Material and methods: Blood and tumour tissue samples were collected from 210 sporadic ccRCC patients and genomic DNA was sequenced using Sanger sequencing. Variants were annotated using in-silico tools and classified based on ACMG guidelines. Protein-protein docking and flexibility analysis were employed to study the interaction between wild-type and mutated VHL models with HIF1A and Elongin C. Statistical analysis was conducted to correlate VHL risk variants with clinical parameters and patient outcomes.

Results: Thirty VHL variants were identified across 171 patients, including seven novel missense mutations, 23 previously reported SNVs, and three novel non-coding variants. In silico analysis showed that mutations negatively impacted pVHL stability and weakened VHL-HIF and VHL-Elongin C interactions. The intronic variant rs61758376 G > C and 3′UTR variant rs1642742 A > G were found to be significantly associated with an increased risk of ccRCC ($OR = 1.676$, $p = 0.0074$; $OR = 1.735$, $p = 0.0171$, respectively). rs1642742 GG genotype was also significantly associated with larger tumour size ($p < 0.05$), advanced tumour stage (pT4) and reduced overall survival (Kaplan Meir log-rank $p = 0.029$).

Conclusion: This study provides novel genetic data from an understudied population and links VHL variants with ccRCC risk and clinical outcomes.

C.O.I. Disclosure:

S. Chatterjee: None. **M. Das:** None.

P01.080.D Genetic characterisation of CSC-associated mutations in prostate cancer: biomarkers on chromosome 17q24-25

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Background: Cancer stem cells (CSCs) are critical drivers of tumor heterogeneity, fueling disease progression and therapy resistance. Prostate cancer (PC), characterized by its variability in clinical outcomes, presents an urgent need for biomarkers linked to CSCs. Prior cytogenetic studies highlighted recurrent alterations in chromosome 17q24-25 within CSC-enriched cultures, suggesting a key role in PC.

Material and methods: To explore this genomic hotspot, we designed a targeted capture panel covering 11 subregions and performed next-generation sequencing (NGS) on CSC-enriched and non-enriched prostate cancer cell lines (PC3 and LNCaP), alongside patient-derived solid biopsies and controls. Subsequent bioinformatic analyses aimed to pinpoint mutations uniquely associated with these populations, distinguishing them from variants found in non-enriched cultures and common genetic backgrounds.

Results: Bioinformatic analysis first identified a unique set of CSC-associated variants present in CSC-enriched cell line cultures. These variants were then investigated in patient-derived prostate cancer biopsy samples, leading to the identification of a panel spanning 13 genes. Among these, four genes (*GRB2*, *PRKCA*, *RPTOR*, and *PRKAR1A*) are particularly notable due to their well-established roles in insulin and mTOR pathways. These genes play crucial roles in pathways closely associated with prostate cancer biology and interact functionally with one another, underscoring their potential as biomarkers.

Conclusion: This study lays the groundwork for identifying CSC-specific genetic patterns, with promising implications for the early diagnosis, classification, and prognosis of PC. Future work will focus on validating these markers and assessing their clinical utility, aiming to bridge the gap between CSC biology and personalized cancer management.

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P01.082.B The c-Maf-inducing protein (CMIP) as a regulator of cancer progression

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Background: The c-Maf-inducing protein (CMIP) is a relatively understudied adapter protein. Several studies showed that CMIP is involved in poor cancer prognosis in several cancer types, such as triple negative breast cancer, ovarian, lung and brain cancer. Nevertheless, the exact underlying mechanisms remain unclear. One study showed that *CMIP* knockdown or overexpression correspondingly affect the expression of *MDM2*, which has previously been identified as an oncogene since it is a known regulator of the tumor suppressor p53. We aim to elucidate the mechanism by which *CMIP* expression regulates cancer progression.

Material and methods: To investigate whether CMIP leads to cancer cell proliferation, we performed a colony formation assay on the MCF7 breast cancer cell line. We tested the effect of CMIP overexpression (using plasmid transfection) and downregulation (using siCMIP) on MDM2 and p53 downstream pathways. Additionally, we conducted RNA-seq analysis on MCF7 cells with either overexpressed or siCMIP to gain an unbiased overview of affected pathways.

Results: Colony formation assay revealed that CMIP down-regulation inhibits cancer cell proliferation. We further investigated whether the pathway involved was related to MDM2-p53. While CMIP overexpression led to an upregulation of MDM2, it did not influence p53-related genes under different cancer-promoting conditions. RNA-seq analysis revealed that siCMIP-treated cells exhibit a notable downregulation of interferon type I genes and a reduction in autophagy-related genes.

Conclusion: CMIP might regulate autophagy, which is known to promote cancer cell survival, through interferon signaling. Determining the role of CMIP in cancer development can unlock new candidate targets for treatment.

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P01.083.C Exposing the DNA methylation-responsive compartment of the leukemic genome supports its potential as a novel therapeutic target in T-ALL

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Background: T-cell acute lymphoblastic leukaemia (T-ALL) exhibits exceptionally high global levels of DNA methylation, with the silencing of the DNA demethylating enzyme TET2 implicated in T-ALL's hypermethylation phenotype. We propose that hypomethylating agents (HMA) could be particularly potent in T-ALL patients with this hypermethylation phenotype. Despite the use of HMAs in treating other hematological malignancies, the role of altered DNA methylation in T-cell cancer biology and its potential as a therapeutic target in T-ALL remain unresolved.

Material and methods: Here we used a novel, reversible DNMT1-specific inhibitor and conventional HMAs to assess the effects of global DNA methylation loss in leukemic cells, integrating toxicity studies, whole genome DNA methylation, and total RNA-sequencing.

Results: We demonstrate that extensive removal of DNA methylation, even in the absence of DNA damage, results in cell death, and that toxicity is negatively correlated with global levels of DNA methylation. Notably, whereas DNA demethylation caused limited transcriptional changes globally, key tumour suppressor genes, such as TET2 and PAX5, were upregulated in a methylation-dependent manner. Few endogenous retroviruses were reactivated after demethylation, and no profound dysregulation of immune-related genes was observed, challenging the contribution of 'viral mimicry' to the observed toxicity.

Conclusion: These findings provide fundamental insights into the role of DNA methylation in cancer, clearly demonstrating that the removal of DNA methylation alone is sufficient to induce cell death in cancer cells. Furthermore, the distinct upregulation of silenced tumour suppressor genes has important implications for

future therapeutic strategies targeting the unique DNA methylation phenotype in T-ALL.

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P01.084.D *TP53BP1* germline variants are associated with hereditary serrated polyposis syndrome

Xavier Dominguez-Rovira[1], *Karin Wallander*[2], *Jenifer Muñoz*[1], *Aina Aguayo*[1], *Marta Pareja-Romagosa*[1], *Yasmin Soares de Lima*[1], *Marcos Díaz-Gay*[3], *Teresa Ocaña*[1], *Sabela Carballal*[1], *Miriam Cuatrecasas*[4], *Guerau Fernàndez*[5], *Isabel Spier*[6], *Anna Sommer*[6], *Stefan Aretz*[7], *Luis Bujanda*[8], *Gabriel Capellá-Munar*[9], *Laura Valle*[9], *Joaquín Cubiella*[10], *Daniel Rodríguez-Alcalde*[11], *Silvia Carles-Gonzalez*[12], *Fatima Perez-Rial*[12], *Clara Ruiz-Ponte*[13], *Antoni Castells*[1], *Francesc Balaguer*[1], *Leticia Moreira*[1], *Sergi Castellvi-Bel*[1], *Laia Bonjoch*[1]

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Background: Serrated polyposis syndrome (SPS) is a clinical condition characterized by multiple and/or large serrated polyps associated with higher colorectal cancer risk (1). Tumor protein p53 binding protein 1 (TP53BP1) is involved in DNA damage response, cell cycle regulation and senescence (2). It was previously suggested as a candidate for SPS germline predisposition(3). The aim of our study was to evaluate *TP53BP1* as a hereditary gene for SPS by performing a functional characterization.

Material and methods: Initial sequencing was performed in SPS cohort of 16 families (39 patients) and validated in 211 SPS cases to find rare, potentially pathogenic genetic variants. We produced CRISPR/Cas9 knock-in cellular models for *TP53BP1* (HAP1 and HCT116 cells) for four genetic variants. We assessed the recruitment of proteins involved in DNA damage response by immufluorescence, overall DNA damage levels by ELISA, and changes in gene expression of senescence markers (*CDKN2A*, *CDKN1A* and *TP53*) by quantitative real-time PCR.

Results: Three rare, predicted pathogenic variants were identified within the *TP53BP1* gene in the discovery and validation cohorts. Four additional variants were found in two external SPS cohorts (4, S.Aretz pers. comm.) Functional characterization of variants revealed significant differences in all three markers of senescence in HAP1 cells carrying the identified genetic variants when compared with the wild-type sequence.

Conclusion: Cells carrying the detected *TP53BP1* variants showed an escape from senescence when compared to cells with wild-type or a neutral variant. Therefore, *TP53BP1* can be postulated as a new hereditary gene for SPS germline predisposition.

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P01.085.A Diagnostic and prognostic molecular classification of pediatric Ependymoma in Italy

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Background: Ependymomas (EPD) are classified according to WHO-2021 by histopatological, molecular and anatomic site features. We provide data on the diagnostic and prognostic markers analyzed in the initial series of 80 Italian pediatric EPD patients enrolled in current SIOP-Ependymoma II clinical trial.

Material and methods: Molecular classification was based on FFPE primary tumor samples. Methylation profiling was performed using Infinium MethylationEPICv2.0 arrays. Fusion genes were analyzed by real time-PCR and sequencing. The TERT expression was analyzed by real time-PCR. Copy-number data were inferred from methylation profiling and CGH-Array.

Results: Methylation profiling was successful in 90% of cases. The CNV/CGH-Array analyses revealed relevant candidate prognostic alterations, including chromosome 1q gain, 6q loss and CDKN2A homozygous loss. In supratentorial EPD cases, the ZFTA-RELA fusion genes was identified in 18/31 cases and the YAP1-MAMLD1 fusion was identified in 2/31 cases. The TERT expression was significantly associated with the presence of RELA fusion. The presence of fusion genes was occasionally associated with chromoanagenesis events. In one fusion-negative supratentorial case, a EWSR1 rearrangement with a breakpoint in intron 7 was revealed.

Conclusion: Molecular diagnostic and prognostic analyses of Ependymoma according to WHO 2021 are feasible and straightforward. The ZFTA-RELA is the most frequent fusion gene and is associated with TERT overexpression. A sample was reclassified as a rare EWSR1-fused brain tumor. Upfront evaluation of prognostic markers will allow a prospective validation in the frame of ongoing SIOP-EPD II trial, in order to inform the molecular stratification strategies for future trials.

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P01.086.B Assessment of the 2022 second revision of the International Staging System (R2-ISS) in patients with newly diagnosed multiple myeloma

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Background: Multiple myeloma(MM) is a hematologic disease with heterogeneous outcomes. In 2022, the Second Revision of

the International Staging System(R2-ISS) was published by the European Myeloma Network(EMN). In this study, we applied R2-ISS to MM patients in Korea and compared R2-ISS to the R-ISS.

Material and methods: Total 489 patients(M:F = 272:217) with newly diagnosed MM between 2015 and 2023 were studied. Bone marrow examinations, chromosomal analysis, FISH, and laboratory tests were performed at the time of diagnosis. All patients were classified according to the R-ISS and R2-ISS. A landmark survival analysis was performed on the 2-year survival rate.

Results: According to the R-ISS, the patients were classified to 55(11.2%) in risk group I, 323(66.1%) in II, and 111(22.7%) in III. The patients were re-classified into 4 risk groups according to R2-ISS, group I: 33 patients(6.7%), II: 104 patients(21.3%), III: 253 patients(51.7%), and IV: 99 patients(20.2%). 23 patients(41.8%) in R-ISS group I were re-classified to R2-ISS group II, 220(68.1%) and 21 patients(6.5%) were re-classified to R2-ISS group III and IV. According to the landmark survival analysis, the 2-year survival rate of patients in R2-ISS group I: 89.5%, II: 83.9%, III: 68.4%, and IV: 46.3%. The differences among the R2-ISS groups were statistically significant($p < 0.05$).

Conclusion: We observed the patients were clearly divided into higher-risk(III, IV) and lower-risk groups(I, II). R2-ISS is an updated risk stratification system and is expected to be a useful tool for predicting patient prognosis and planning treatment.

C.O.I. Disclosure:

S. Min: None.

P01.087.C Brain MRI surveillance of optic pathway glioma in pediatric neurofibromatosis Type 1: findings from a Cohort of 61 patients

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Background: Children with neurofibromatosis type 1 (NF1) rarely die from optic pathway gliomas (OPG) but may experience vision loss, proptosis, hydrocephalus, precocious puberty, and social or daily life difficulties. There is no standardized method for monitoring OPG in pediatric NF1 patients. Although annual ophthalmological examinations (OE) are recommended, brain MRI for pre-symptomatic OPG detection is optional. This study evaluates the effectiveness of MRI compared to OE in diagnosing OPG through an ambispective analysis of 61 children from 2013 to the present.

Materials and methods: We reviewed medical records retrospectively and prospectively. In the prospective phase, brain MRI was performed at diagnosis or at 18 months, followed by scans every two years until age eight and again at 18 years. All participants underwent annual OE. RESULTS Of the 61 patients, 21 (34%) had OPG. Six had OPG before their initial clinic visit, with the status of five unknown. Among the remaining 50 patients, 15 (30%) were diagnosed via MRI at a median age of 2 years. Three patients required chemotherapy due to complications; one with chiasmal OPG had normal OE results before MRI detected progression. Two patients reported vision difficulties, with abnormal OE preceding MRI confirmation. Chemotherapy began at ages 4, 5, and 7, but one patient did not regain vision post-treatment.

Conclusion: Our findings suggest that MRI is more effective than OE in detecting OPG. Early detection through MRI surveillance alongside OE is critical to preventing vision loss. Further studies are needed to determine the optimal frequency of MRI surveillance.

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P01.088.D Investigating the tumor microenvironment through single-cell transcriptomics in pre- and post-chemotherapy neuroblastoma patients

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Background: Neuroblastoma (NB) is the most common extracranial solid tumor in children, with high-risk cases still showing poor outcomes despite improved therapeutic protocols. Tumor microenvironment plays a pivotal role in resistance to therapy, and specific cell types, e.g., cancer-associated fibroblasts (CAFs) might be involved in this process. Here we performed single-cell RNAseq (scRNAseq) on NB biopsies before and after treatment to investigate microenvironment changes at the single cell resolution.

Material and methods: Matched (pre/post chemotherapy) biopsies (n = 33) from 18 patients were processed with the Gexscope kit. Expression data was imported in R and analyzed with the Seurat pipeline. Non-tumor cells were selected according to multiple references and the absence of copy number variation. Specific cellular subtypes were identified based on the presence of specific markers from literature.

Results: A total of 23,457 non tumor cells were identified, mainly composed of immune (35%) and Schwann cells (23%). We found an increase of Schwann cells in post-chemo cells compared to pre-chemotherapy cells (35.8% vs 1.7%), also myeloid cells were increased after chemotherapy (22.4% vs 17.3%), while B- and T-cells were decreased. Focusing on the fibroblast subtypes, we found 1458 CAFs in pre-chemotherapy samples and 2332 CAFs in post-chemotherapy samples. Although this difference was not significant, we identified specific CAF subtypes, e.g., inflammatory CAFs, involved in tumor progression and immunosuppression.

Conclusion: scRNAseq is a powerful tool to investigate differences in tumor microenvironment at single-cell resolution. Further analysis on other cell subtypes (e.g., immune cells) are currently ongoing.

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P01.089.A Multi-omic characterization of hereditary prostate cancer: *AXIN2* and *DICER1* mutations in tumor development

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Background: Only 5%–10% of all prostate cancer (PC) cases are hereditary. Existing clinical guidelines, such as those from the National Comprehensive Cancer Network, are insufficient for diagnosing and stratifying these patients due to the heterogeneity and complexity of this cancer. Present study aims to identify genetic factors contributing to hereditary PC through a multi-omic approach.

Material and methods: A multi-omic analysis was conducted on a Spanish multi-case PC family, incorporating exome, transcriptome, and epigenomic profiling. Data integration encompassed microRNA-gene interactions and methylation-gene associations to provide a holistic molecular perspective.

Results: Analysis of 34 genes, selected based on clinical guidelines and literature, revealed 268 variants in 26 genes, predominantly intronic (91.4%) and unreported (84.3%). While no pathogenic mutations were identified in genes recommended by clinical guidelines, ten identical mutations were detected in *AXIN2*, *DICER1*, and *BARD1* across all PC cases studied. Due to the critical roles of *AXIN2* and *DICER1* in the Wnt signaling pathway, our focus led to the identification of a shared intronic mutation in the nonsense-mediated decay β-catenin isoform. Transcriptomic analysis revealed *ALDH1A1* overexpression, a well-established Wnt/β-catenin target gene, while epigenetic studies indicated differential DNA methylation and miRNA expression linked to key oncogenic pathways.

Conclusion: Our study underscores the role of intronic mutations in PC development and highlights the potential of incorporating *AXIN2* and *DICER1*, due to their important role in Wnt signaling pathway, in clinical gene panels for better risk assessment. Further research in additional families is crucial to confirm these findings and improve genetic screening methods.

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P01.090.B Resolving discordant tumor and germline testing results in MMR-deficient Carcinomas: paired testing approach to distinguish Lynch and Lynch-like syndromes

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Introduction: Lynch syndrome(LS) is the most common hereditary cancer predisposition syndrome accounting for ~3% of all colorectal and endometrial carcinomas. First-line approach to differentiate between LS and sporadic cancer is immunohistochemical(IHC) staining to assess MMR protein expression in tumor tissue. Reflex genetic testing after an abnormal IHC result includes tumor *MLH1* methylation and/or BRAF V600E, then targeted germline testing. However, MMR IHC might be impaired without underlying MMR hypermethylation,BRAF alteration, or germline MMR pathogenic variant(PV). Differentiating "Lynch-like syndromes" is essential for patient management and cascade counseling.

Methods: We examined the results of paired tumor *MLH1* hypermethylation/BRAF mutation and comprehensive germline testing in a cohort of 51 individuals with Lynch-associated tumours showing abnormal MMR IHC ("mismatch repair deficient = MMRd").

Results: Among 51 MMRd patients, 68% (34/51) had MMR hypermethylation; including one with constitutional MLH1 hypermethylation. 44 patients [29/44 MMR hypermethylation, 15/44 normal MMR-methylation] were available for germline testing by NGS panel [Illumina TruSight Cancer/TruSight Hereditary Cancer Panel] and dijital MLPA analysis [MRC-Holland D001-B1/C1]. The overall rate of inconclusive results was 27% (12/44), including one patient with *MLH1* hypermethylation and germline *MSH6* PV, one with *MLH1* hypermethylation and germline *SMARCA4* PV, two with normal methylation and germline PV in non-MMR genes (*NBN, BARD1*). Notably, eight MMRd patients had negative tumor methylation/germline tests, indicating potential false negatives in IHC (8/44).

Conclusions: Our findings indicate that IHC-guided testing alone may miss germline mutations in other MMR and non-MMR genes, reinforcing that the ideal strategy for achieving a higher diagnostic yield for LS is paired tumor and germline testing.

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P01.091.C Spectrum of germline and somatic variants in the *DICER1* gene in pediatric patients with DICER1-specific tumors

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Background: DICER1 syndrome is characterized by predisposition to a tumor growth during the lifetime and caused by germline pathogenic variants (PVs) in the *DICER1* gene. We present the results of germline and tumor tissue genetic testing in children with DICER1-associated malignant and benign tumors during a 6-year period in our Center (2019–2024).

Material and methods: The retrospective analysis of 29 probands aged 9 month-18 years old with a variety of solid tumors suspected of DICER1 syndrome were evaluated. The molecular genetic testing methods included a panel-based and whole genome NGS, MLPA and Sanger sequencing.

Results: Among 29 probands suspected of DICER1 syndrome, the germline PVs in the *DICER1* gene were confirmed in 19 probands. One proband had PV (c.5426G>T/p.G1809V) in one of the hotspot codons in the RNase IIIb domain, inherited from a mosaic father. The germline PVs were not found in 10/29 (34,4%) probands. The spectrum of the tumors in these 10 patients included pleuropulmonal blastoma (5 cases), cystic nephroma (2 cases), Sertoli-Leydig cell tumor (2 cases) and embryonal rhabdomyosarcoma (1 case). In 3/10 cases two *DICER1* variants in the tumor tissue were detected, but for none of them the germline status was confirmed.

Conclusion: About two-thirds of probands suspected of DICER1 syndrome had germline PVs in the *DICER1* gene. Further studies are needed in those 3 cases when two variants were found to determine if they are indeed originated from tumors only, or if those cases may reflect a somatic mosaicism.

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P01.092.D Beckwith-Wiedemann Spectrum and acute lymphoblastic leukemia

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Background: Beckwith-Wiedemann Spectrum (BWSp) is a cancer predisposition syndrome primarily associated with embryonal tumors such as nephroblastoma and hepatoblastoma. Its correlation with hematological malignancies, particularly Acute Lymphoblastic Leukemia (ALL), is unclear. ALL is the most common pediatric cancer, with a cumulative risk of 0.05–0.07% before 18 years. Extremely limited data, based on single reports, suggest an ALL incidence of ~0.5% in BWSp patients with chromosome 11 paternal uniparental disomy (UPD(11)pat) and ~1.4% in those CDKN1C-mutated.

Material and methods: We report two new cases of ALL in BWSp patients and review the existing literature.

Results: The literature review identified six cases of ALL in BWSp patients (3 males), with a mean age at diagnosis of 8.2 years (range 2–21). Among them, two had CDKN1C variants, one UPD(11)pat, one IC2-Loss of Methylation (LoM) due to a 11p15 microdeletion, and two were untested. Two had T-ALL, three B-ALL, and one was unspecified. One B-ALL case was Philadelphia-positive. Additionally, we report a male with mosaic UPD(11)pat who developed B-ALL at 6 years and a female with IC2-LoM diagnosed with Philadelphia-positive B-ALL at 4.8 years.

Conclusion: T-ALL represents 10–15% of pediatric ALL but accounted for 2/7 (29%) of BWSp-ALL cases. Similarly, Philadelphia-positive B-ALL is rare in pediatric patients (3–5%) but accounted for 2/4 (50%) of BWSp-B-ALL cases, suggesting a possible overrepresentation in the BWSp context. The currently available data are too limited to draw definitive conclusions. Further studies are needed to determine ALL incidence, subtype distribution, treatment response, and toxicity in BWSp patients

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P01.093.A Detection of germline mutations in patients with cutaneous melanomaby universal genetic testing*vs*guideline based testing and identification of novel potential relevant genes

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Background: The most frequently implicated gene in familial melanoma is ***CDKN2A***, accounting for 20%–45% of cases, while other risk genes include ***MITF, CDK4, TERT, POT1*** **and** ***MC1R***. Current guidelines recommend screening for patients meeting specific criteria: family history of Cutaneous Melanoma (CM), multiple primary melanomas, a history of CM-associated cancers. However, these criteria may fail to detect clinically relevant mutations in some individuals, potentially impacting cancer prevention strategies. This study aimed to assess the utility of extending genetic testing to CM patients who do not meet standard eligibility criteria.

Material and methods: Between June 2019-May 2024, **790 patients** were recruited, with **244** fulfilling the established criteria **(group 1)** and **546**not meeting them (**group 2**). A **multigene panel** was used to analyze CM-associated genes along with genes associated to other cancer syndromes (e.g. **BRCA1-2, CHECK2, ATM**).

Results: The results showed that **31.9% of group 1 patients (N = 78)** carried **pathogenic/likely pathogenic (P/LP) variants**, compared to **6.4% (N = 35) in group 2**. Six patients carried **P/LP variants** in genes related to other cancer syndromes, whose correlation to CM requires further investigation. For five negative patients, that strongly met inclusion criteria, **exome equencing** was performed revealing **P/LP in four cases** in genes potentially associated with melanoma, namely ***FLG, CDH13, MAP2K2*****, and** ***PHF10***.

Conclusion: These findings suggest that expanding genetic testing criteria for CM patients can help identify individuals carrying significant mutations that would otherwise go undetected. Considering the benefits of early diagnosis and cancer prevention, the cost-effectiveness of universal genetic screening should be further explored.

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P01.094.B Investigation of Lynch-related variants in breast cancer cases: is there an overlooked association?

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Background: Lynch syndrome is one of the most prevalent hereditary cancer syndromes. Its prevalence is approximately 1 in 279. While many studies have suggested a modest increase in breast cancer risk, the relationship between breast cancer and Lynch syndrome remains controversial. This study aims to enhance our understanding of the potential association between Lynch syndrome and breast cancer through an analysis of our own data.

Material and methods: Next-generation sequencing panel data, including hereditary cancer predisposition genes, of 861 patients diagnosed with breast cancer between 2018 and 2024 were collected retrospectively. Variants classified as pathogenic (P), likely pathogenic (LP), and variant of uncertain significance (VUS) in Lynch syndrome-associated genes were re-analyzed.

Results: Among the 861 patients, 84 P/LP and VUS variants were identified in 81 individuals. In the *MLH1* gene, 3 LP variants (0.35%) and 37 VUS (4.30%); in the *MSH2*, 1 LP variant (0.12%) and 14 VUS (1.63%); in the *MSH6*, 28 VUS (3.25%) and in *PMS2* gene, 1 VUS (0.12%) were detected. Overall, LP variants were observed in 0.46%, VUS were observed in 9.29% of the patients. Patients with detected likely pathogenic variants did not have colorectal or endometrial cancer history.

Conclusion: When compared to the population prevalence of Lynch syndrome, the frequency of LP/P variants identified in the breast cancer cohort was found to be similar. In cases where breast cancer is the only symptom, Lynch syndrome should be considered only after the exclusion of primary breast cancer associated gene mutations.

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P01.095.C Metabolic dependencies in esophageal adenocarcinoma: exploring *TP53* mutation-driven phenotypes for targeted therapies

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Background: Esophageal adenocarcinoma (EAC) is an aggressive malignancy with a rising incidence and poor prognosis. High genomic heterogeneity in EAC hinder prevention, early diagnosis and targeted therapies development. *TP53* is the most frequently mutated gene, with missense mutations strongly linked to reduced cancer-specific survival. This study aims to identify novel molecular vulnerabilities by analyzing the metabolism of three in-vitro cell models representative of different stages of tumor progression and with distinct *TP53* mutations: OE33 and FLO-1 cells carry missense variants (p.Cys135Tyr and p.Cys277Phe, respectively), OE19 cells carry a frameshift variant (p.Asn310Lysfs*27).

Methods: Live cell imaging and recording, ATP/ADP and lactate quantification, real-time PCR were used on the different cell lines.

Results: OE33 cells predominantly use oxidative metabolism but display limited adaptability to environmental changes, maybe due to a higher differentiation state *vs*. the other cells. OE19 cells are mainly dependent on oxidative metabolism, showing adaptability to glucose and glutamine deprivation. They use galactose as alternative energy substrate due to increased *GALE* expression and their growth is impaired by electron transport chain inhibitors, i.e. Antimycin A ($P = 0.0133$). FLO-1 cells rely on glycolytic metabolism, with elevated glucose consumption and lactate production, marked *GAPDH* expression ($P < 0.0001$) and glutamine-dependent proliferation. Their growth is unaffected by acidic conditions (pH 6.8), as observed in tumor niches.

Conclusion: We identified unique metabolic phenotypes in three in vitro models of EAC, possibly driven by distinct TP53 mutations. Targeting these specific metabolic vulnerabilities can yield insights for personalized therapies, improving treatment outcomes for EAC patients.

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P01.096.D Murine migration-stimulating factor ortholog: insights into FN1 splicing and cancer

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Background: Fibronectin 1 (FN1) is a key extracellular matrix (ECM) glycoprotein involved in cell adhesion, migration, and tissue remodelling. Among its isoforms, the Migration-Stimulating Factor (MSF) is a truncated oncofetal variant with potent motogenic properties, implicated in cancer progression and metastasis. Despite extensive research on human MSF, the lack of a murine ortholog has limited preclinical investigations. This study identifies and characterizes the murine MSF ortholog, elucidating its expression, regulatory mechanisms, and functional relevance in cancer biology.

Materials and methods: We identified murine MSF through alternative polyadenylation analysis in FN1. 3′ RACE and Sanger sequencing were used to reconstruct its transcripts, followed by qPCR to assess expression in murine tissues and pancreatic cancer cells (PANC02). Functional validation included gapmer-mediated silencing, proliferation assays, and RNA-seq. The secretion pathway is under investigation using the RUSH system.

Results: We identified two murine MSF isoforms with distinct 3′-UTRs, analogous to human MSF. Expression analysis showed MSF overexpression in PANC02 cells compared to non-tumorigenic controls. Silencing experiments led to a significant reduction in proliferation (−40%). Preliminary RNA-seq analysis suggests potential downregulation of ECM remodeling and growth factor signaling genes in MSF-silenced cells, indicating a possible role for MSF in modulating these pathways. Additionally, we identified circRNA involvement in MSF regulation, suggesting a post-transcriptional control mechanism.

Conclusion: Murine MSF is functionally analogous to human MSF, promoting tumorigenic processes through. Its regulation via alternative polyadenylation and circRNAs highlights its role in ECM dynamics. These findings provide a foundation for future investigations into MSF-targeted cancer therapies.

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P01.097.A Identification of biological process involved in bladder cancer through multi-omics integration

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Background: Bladder cancer (BLCA) is the 9th most prevalent cancer, and its heterogeneity in clinical outcomes necessitates a deeper understanding of its underlying molecular mechanisms. This study leverages multiomics data of 120 individuals from the

Torino Bladder Cancer Study (TBCS) to explore molecular pathways involved in BLCA.

Methods: TBCS included histologically confirmed BLCA patients (enrolled 1994–2007) from Turin, with non-cancer controls that were matched for age, sex, ancestry, and smoking status. We analyzed peripheral blood data from 120 individuals (58 cases, 62 controls) who have been subjected to genome-wide genotyping, mRNA profiling, and DNA methylation profiling. Single-omics analyses were conducted using PLINK2 and the R packages minfi, ChAMP, and limma, and then integrated using Multi-omics factor analysis (MOFA). For the MOFA analysis, we selected 64 pruned SNPs from the GWAS, along with the residuals of 951 differentially methylated CpGs and 144 differentially expressed genes ($P < 0.05$), to serve as features in the model.

Results: MOFA identified 15 factors that accounted for the variability within the data, with gene expression and methylation CpGs explaining >40% of total variance. Factor 1 showed the strongest correlation with disease status, tumour grade and number of recurrences. Enrichment analysis showed that the top 30 features that carried the most weight were predominantly involved in the Interleukin-2 and Interleukin-12-mediated signaling pathways, suggesting their roles in disease pathogenesis.

Conclusions: This analysis provides a systematic framework to investigate BLCA pathobiology, potentially reflecting both tumor-derived and host immune responses through a multiomics analysis.

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P01.098.B CD39 expression and STAT3 activation in Sézary Syndrome: insights into genetic regulation and potential therapeutic targets

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Background: Sézary Syndrome (SS) is an aggressive cutaneous T-cell lymphoma characterized by clonal CD4+ T-cell expansion, clinical heterogeneity, and unclear molecular and genetic mechanisms. We identified aberrant expression of CD39 and CD73 in SS CD4+T lymphocytes, leading to accumulation of immunosuppressive adenosine, which may drive phenotypic variability in SS patients.

Material and methods: A cohort of 20 SS patients was genotyped for the SNPs rs10748643 (A > G) and rs7096317 (G > A) in the ENTPD1/CD39 gene using Sanger sequencing, combined with multiparametric flow cytometry. PBMCs from SS patients and HUT-78 cell line were used for functional assays (Western blot, PrestoBlue, CFSE).

Results: CD39 expression is genetically regulated by rs10748643 and rs7096317, which are in perfect linkage disequilibrium ($r^2 = 1$). Homozygous individuals for rs10748643G and rs7096317A expressed the highest CD39 levels, particularly in CD4+ SS cells and Treg cells, but not in other cell subsets. CD4+ SS cells showed reduced proliferation upon stimulation through TCR or IL-2 and unmodified CD39 expression regardless of genotype. At the molecular level, both circulating CD4+ SS cells and HUT-78 cells exhibited high levels of phosphorylated STAT3, a key transcriptional factor in the JAK/STAT pathway, particularly in CD39+ SS cells, along with enhanced mTOR activation and MCL-1 upregulation. STAT3 inhibition with Stattic-V reduced phospho-STAT3 levels, decreasing cell survival and CD39 expression, suggesting a role for STAT3 in CD39 transcriptional regulation in SS.

Conclusion: The genetic regulation of CD39 and its interaction with STAT3 signaling provide novel insights into SS pathogenesis, supporting potential therapeutic strategies targeting CD39 and STAT3 in SS.

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P01.099.C Pathogenic variants in cancer genes in the Slovenian population

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Background: Reporting secondary findings is recommended for genes associated with hereditary cancer[1]. The proportion of pathogenic variants in cancer-related genes in human populations varies significantly[4,3], therefore population-specific results are needed to help assess genomic risks and disease burden in the given population and aid in public health genomic development[4].

Methods: All rare loss of function variants and known pathogenic variants in ClinVar in the 27 dominant hereditary cancer-related genes of the ACMG Secondary Findings v3.2 list1 were collected from the genomic data of the 14,000 individuals in the Slovenian genome database. The variants were classified according to the ACMG criteria[5] and the population burden of (likely) pathogenic findings was estimated.

Results: The (likely) pathogenic variants were most frequently found in the *BRCA1* and *BRCA2* genes (0.34% and 0.32% respectively), followed by *PALB2* (0.14%), *PMS2* (15%), *MSH6* (0.08%) and *RET* (0.07%). The burden of pathogenic variants in *APC*, *MEN1*, *MLH1*, *MSH2*, *NF2*, *PTEN*, *RB1*, *SDHAF2*, *SDHB*, *SDHD*, *SMAD4*, *TMEM127*, *VHL* and *WT1* was between 0.03% and 0.01%. No pathogenic variants were found in *BMPR1A*, *MAX*, *SDHC*, *STK11*, *TSC1*, *TSC2*, and *TP53* in our unaffected population. The cumulative prevalence of pathogenic variants in hereditary cancer-related genes in the Slovenian non-cancer population was 1.27%.

Conclusion: The proportion of (likely) pathogenic variants in the Slovenian population in the medically actionable genes, associated with hereditary cancer, is 1.27%. The highest proportion is in genes, associated with hereditary breast and gynecologic cancer, as we reported previously[6].

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P01.100.D Collecting multifactorial breast cancer risk information: evaluating the MyCanRisk tool

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Background: The MyCanRisk tool is a platform-independent application designed to support the collection of information required for multifactorial risk assessment using the CanRisk tool (www.canrisk.org). It enables patients to provide information about personal risk factors before a healthcare professional completes the risk assessment, streamlining the assessment process. This study evaluates MyCanRisk's efficacy, user-friendliness, and alignment with existing clinical methods.

Material and methods: A mixed-methods study was conducted with 51 women (aged ≥ 18) who had a family history of breast cancer but no personal cancer diagnosis. Participants completed the MyCanRisk tool and, two weeks later, underwent a structured interview modelled on best practices in clinical genetics. Risk factor information from both methods was compared and Cohen's Kappa (κ) was used to evaluate consistency in risk categorisation. Reflective interviews with a subset of participants (n = 15) provided qualitative insights into user experience and tool usability which were analysed thematically.

Results: The agreement between MyCanRisk and structured interviews for risk categorisation was substantial (κ = 0.81, 95% CI 0.65–0.97). The MyCanRisk tool was judged to be usable, with participants highlighting its clarity and flexibility. Some participants shared difficulty recalling details, mixed emotional impacts, and provided suggestions for enhanced guidance, consistent with existing challenges in collecting family history and conducting multifactorial risk assessment in clinical practice.

Conclusion: MyCanRisk is a user-friendly and effective tool for collecting risk factor information, and offers the opportunity to streamline breast cancer risk assessment using CanRisk. These findings support the further integration of MyCanRisk into clinical and research settings.

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P01.101.A Genetic profiling for renal cell carcinoma risk assessment: multi-SNP analysis in a spanish cohort

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Background: Renal cell carcinoma (RCC) is a heterogeneous malignancy influenced by genetic and environmental factors. Genome-wide association studies (GWAS) have identified several RCC susceptibility SNPs within key genes, including *ITPR2, DPF3, EPAS1, PVT1*, and *MYC*, which are involved in calcium signaling, chromatin remodeling, hypoxia response, and oncogenesis. Despite their biological relevance, the functional impact of these genetic variations in sporadic RCC remains poorly characterized. This study aims to identify potential genetic biomarkers for early diagnosis in a Spanish cohort.

Material and methods: Buccal swabs from 259 healthy controls and 168 RCC patients were genotyped for key SNPs, and expression analysis was conducted on 30 formalin-fixed, paraffin-embedded renal tissue samples, including both tumor and adjacent healthy tissue. Statistical analyses were performed to evaluate the relationships between genotypes, gene expression, and RCC risk.

Results: The CC genotype of *ITPR2* rs10771279 was associated with a protective effect, with higher *ITPR2* expression observed in healthy tissue compared to RCC. The C risk allele of *DPF3* rs4903064 correlated with increased RCC risk and elevated *DPF3* expression, suggesting a role in hypoxia-inducible pathways. Similarly, *EPAS1* rs7579899 showed a significant association with RCC risk, consistent with its role in hypoxia-regulated pathways. Although *PVT1* rs35252396 did not reach statistical significance, *PVT1* gene expression was significantly upregulated in RCC tissue.

Conclusion: This study identifies key SNPs and genes, including *ITPR2, DPF3*, and *EPAS1*, as contributors to RCC susceptibility, with multi-SNP combinations showing strong predictive potential, underscoring the value of genetic profiling in RCC risk assessment.

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P01.103.C KRAS p.G12C and PIK3CA p.Q546K as tumors biomarkers for reclassifying variants of uncertain significance in MUTYH

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Background: *MUTYH*-associated polyposis (MAP) is an underdiagnosed recessive syndrome that predisposes individuals to colorectal cancer (CRC). Biallelic MUTYH loss leads to a distinct somatic mutational signature, characterized by G > T transversions, frequently resulting in KRAS p.G12C and PIK3CA p.Q546K mutations in CRC and adenomas of MAP patients—mutations rarely observed in sporadic tumors. This study aims to assess the utility of detecting KRAS p.G12C and PIK3CA p.Q546K in adenomas and CRCs for classifying variants of uncertain significance (VUS) in MUTYH.

Material and methods: We analyzed the frequency of KRAS p.G12C and PIK3CA p.Q546K mutations in adenomas and CRC tissues from 16 MAP patients and 3 individuals harboring MUTYH VUS (NM_001128425.2: p.Trp113Arg, p.Pro301Arg, and p.Lys450Met, detected in homozygosity or compound heterozygosity with a pathogenic variant).

Results: Among 17 adenomas from MAP patients, 8 (47%) carried KRAS p.G12C, while none harbored PIK3CA p.Q546K. In 13 CRCs, these mutations were detected in 92% and 38% of cases, respectively. In suspected MAP cases, KRAS p.G12C and PIK3CA p.Q546K were identified in one adenoma and one CRC from the p.Trp113Arg carrier, while KRAS p.G12C was found in one CRC from the p.Pro301Arg carrier. These findings support their classification as likely pathogenic according to ACMG PP4_moderate or PP4_supporting criteria.

Conclusions: The high prevalence of KRAS p.G12C and PIK3CA p.Q546K in MAP-associated CRC and adenomas highlights their potential as biomarkers for classifying MUTYH VUS. This simple approach offers a valuable complementary strategy for variant interpretation, improving the accuracy of genetic diagnoses for individuals with suspected MAP.

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P01.104.D Familial case of ultra rare constitutional mismatch repair deficiency - like syndrome

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Background: The proband was an 8-year-old female with nephroblastoma that manifested at the age of 20 months. The patient also had multiple café-au-lait (CAL) spots, as well as depigmented areas, and microcephaly. Her older sibling had two metachronous tumors: choroid plexus papilloma at the age of 2, and esthesioneuroblastoma at the age of 5 which led to a lethal outcome at the age of 7 years. He also had seizures, speech delay and multiple CAL spots. Both parents were clinically healthy. The diagnosis of constitutional mismatch repair deficiency (CMMRD) syndrome was suspected.

Material and methods: NGS-based sequencing with a target panel of cancer predisposition genes was carried out, with a special focus on *MLH1*, *MSH2*, *MSH6* and *PMS2* genes, but no pathogenic or likely pathogenic variants were detected. The next step was trio whole genome sequencing in both short- and long-read format.

Results: Two rare changes in the *MAD1L1* gene in trans were considered to be of particular interest - c.244C>T/p.Arg82Ter (exon 4) and c.1888C>T/p.Arg630Cys (exon 18). In 2022, the first and the only one to date case of autosomal recessive mosaic variegated aneuploidy (MVA) syndrome 7 with inflammation and tumor predisposition associated with the *MAD1L1* gene was described in a 36-year-old female with a dozen of malignant and benign neoplasms (1).

Conclusion: As far as we know, this case may be the second described case of MVA type 7 in the literature. Further studies are needed to prove the causativity of the variants for the patient's phenotype.

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P01.105.A Long-term chromosomal aberrations in fibroblasts 20 years after total body irradiation

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Background: Total body irradiation (TBI) and prophylactic whole brain radiation therapy (WBRT) can cause long-term radiation effects, such as chromosomal aberrations in skin fibroblasts and white matter changes potentially linked to cognitive decline.

Case study: A 45-year-old female was referred to our genetics clinic with cognitive deficits and cerebrospinal fluid biosignature suggestive of early-onset Alzheimer's. At 26, she was diagnosed with Pre-B-Cell Acute Lymphoblastic Leukemia (ALL) and treated with allogeneic stem cell transplantation requiring TBI-based conditioning with hyperfractionated radiotherapy (12 Gy) and WBRT (24 Gy). Family history included ALL (sister), breast cancer (mother) and lymphoma (maternal grandmother).

Methods: A skin biopsy was obtained and fibroblasts were cultured using standard protocols. Karyotyping was performed on G-banded metaphase chromosomes. Genome sequencing was performed on DNA from cultivated fibroblasts to evaluate possible predisposition to dementia and hematopoietic neoplasms.

Results: Aside from the heterozygous *APOE* ε4 risk allele and a heterozygous variant of unclear significance in *NOS3*, the genome analysis did not reveal any (likely) pathogenic variants. Interestingly, the chromosome analysis showed multiple aberrations in all mitoses of 2 cultures which included multiple reciprocal translocations and inversions together with numerical aberrations. These findings were verified in a second skin biopsy.

Conclusion: No causative variants for chromosomal breakage or hereditary cancer predisposition syndromes were found in the genome analysis (pending verification with LR-genome analysis). Our current findings suggest that the complex chromosomal aberrations possibly resulted from the ALL treatment 20 years ago. Further research regarding a possible causative connection with early-onset dementia is warranted.

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P01.106.B Elucidating the role of human leukocyte antigens in antidrug antibody formation from a large Cohort study of early-phase non-standard cancer immunotherapy trials

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Background: Checkpoint inhibitors (CPIs) have revolutionized cancer treatment, but the development of antidrug antibodies (ADA) can limit their efficacy and increase immune-related adverse events. Identifying biomarkers predictive of ADA formation is critical. Human leukocyte antigen (HLA) genes, known for their role in immune responses, have been linked to ADA formation in CPI therapy. However, the impact of HLA alleles on ADA development in non-CPI cancer immunotherapy (CIT) remains largely unexplored.

Methods: To investigate the association between HLA alleles and ADA formation, we analyzed a harmonized dataset from 23 early-phase clinical trials, encompassing 12 diverse molecules and various cancer indications, including 3'568 patients, both CPI-naïve and CPI-experienced. ADA status was determined using

standardized definitions based on therapeutic antibody-specific titers. We employed an HLA-wide approach to assess associations with persistent ADA formation.

Results: After Bonferroni correction, we found no statistically significant associations with low consistency in the ranking of HLA alleles. However, results for CPI as a standard-of-care combination therapy showed an odds ratio of 1.84 (CI 95% 1.02–3.21) for the DRB1*01:01 variant, consistent with previous findings in atezolizumab monotherapy trials.

Conclusions: This study represents a systematic investigation of the role of the HLA region in ADA formation in non-standard CITs. The low correlation among HLA allele rankings suggests that genetic predisposition to ADA formation is likely molecule-specific. However, these results may be influenced by low statistical power and other confounding factors. Future work will integrate T-cell epitope prediction models to further elucidate the immunogenicity cascade and differences observed across therapeutic antibodies.

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P01.107.C Common and rare variants integration for Breast Cancer risk stratification to personalize risk-adapted preventive measures

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Background: Common low-risk variants are presently not used to guide clinical management of familial breast cancer (BC). Many studies suggest that the combined effects of BC-associated single nucleotide polymorphisms (SNPs) can improve BC risk stratification using polygenic risk scores (PRS). We explored the additive impact of PRS and the potential modulation of the effect of mutations in high/moderate risk susceptibility genes in BC patients.

Material and methods: We analysed the genetic profiles of 195 Italian BC patients using Illumina SNP array and Whole Exome Sequencing (WES) Firstly, we compared two different PRSs, PGS000335 (1,079,089 SNPs) and our new optimized PRS composed by 4606 SNPs. Then, we evaluated the modulation of BC risk by the PRS and the potential combined effects of rare variants using the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm V.5 model (BOADICEA), calculating lifetime risks retrospectively both with and without individual PRS.

Results: The BC patients PRSs distributions were higher compared to controls. *BRCA1/2* variants had a major role in stratifying lifetime risk, while PRS improves risk estimation for patients with variants with an uncertain meaning and non-carriers. Using PRS could also improve the risk prediction for those patients carrying a mutation on gene with a moderate BC risk as *ATM*, *CHECK2*, *BARD1*, *BRIP1, RAD51C* and *RAD51D*.

Conclusion: PRSs may be helpful to personalize risk-adapted preventive measures for women with BC, even when they carry mutations in high/moderate risk genes.

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P01.108.D Long noncoding RNA NEAT1 interacts with the miR-3648 axis in imatinib resistant chronic myeloid leukemia cell model

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Background: Chronic myeloid leukemia (CML) is a hematopoietic malignancy caused by the formation of the Philadelphia chromosome (Ph, 9;22). This results in the expression of the BCR-ABL fusion protein, which possesses tyrosine kinase activity. The leukemogenic nature of BCR-ABL1 makes it a therapeutic target in CML treatment. Imatinib, the first clinically approved tyrosine kinase inhibitor (TKI), is an ATP-competitive inhibitor that specifically binds to the BCR-ABL oncoprotein, suppressing its kinase activity. However, resistance to BCR-ABL tyrosine kinase inhibitors has been observed over time. This resistance can also occur independently of BCR-ABL1 mutations. Therefore, this study aims to investigate the epigenetic regulation of the imatinib resistance mechanism in CML. Long non-coding RNAs (lncRNAs) are RNA molecules ranging from 200 bp to 100 kbp in length that play a role in epigenetic mechanisms and gene expression regulation. The long non-coding RNA NEAT1 is responsible for gene regulation and facilitates the formation of nuclear bodies called 'paraspeckles.'

Material and methods: In this study, RNA pull-down using biotinylated primers specific to NEAT1 and RNA sequencing were performed in the imatinib-resistant K562 cell line to investigate the presence of miRNAs retained in NEAT1/paraspeckles.

Results: miR-3648 was found to be associated with NEAT1 in imatinib-resistant K562 cells compared to imatinib-sensitive K562 cells.

Conclusion: We hypothesize that miR-3648, which is retained within paraspeckles in imatinib-resistant K562 cells, may contribute to the imatinib resistance mechanism by being unable to bind to its target genes, thereby affecting cell survival rates.

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P01.109.A The role of *TET2* mutations and expression in high grade serous ovarian carcinoma: impact on molecular and clinico-pathological features

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Background: High Grade Serous Ovarian Carcinoma (HGSOC) is the deadliest gynecological cancer, with limited understanding of the role of epigenetic mechanisms in its pathogenesis. TET2, an enzyme involved in DNA demethylation, plays a critical role in maintaining epigenomic stability. Although *TET2* alterations have been linked to tumorigenesis and therapy response in other cancers, their role in HGSOC remains poorly understood, highlighting the need to investigate their molecular and clinical implications.

Material and methods: Genomic and transcriptomic analyses of *TP53*-mutated HGSOC samples were conducted using AACR GENIE (n = 3641) and TCGA GDC (n = 222) datasets. Genomic analysis examined *TET2* mutations and their associations with demographic and clinical parameters, while transcriptomic analysis evaluated correlations between *TET2* expression and therapy resistance pathways. Statistical methods included Spearman correlation, Chi-square tests, and Kaplan-Meier survival analysis.

Results: *TET2* mutations were identified in 6% of patients, predominantly among Black and Asian populations, with missense mutations being the most frequent. These mutations correlated with alterations in the therapy resistance pathway. Transcriptomic analysis revealed higher *TET2* expression in Black and Asian populations compared to White counterparts, correlating strongly with mRNA levels of therapy resistance genes and a trend toward worse overall survival.

Conclusion: These preliminary findings suggest that *TET2* mutations and expression may influence therapy response and survival in HGSOC. Further validation is required to confirm these observations and clarify the mechanisms underlying *TET2*'s role in therapy resistance. Understanding these processes could pave the way for developing TET2-based biomarkers and personalized therapeutic strategies, ultimately improving outcomes for patients with *TET2* alterations.

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P01.110.B DMU-212 potently and selectively targets leukemia stem cells in vitro

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Background: Leukemia stem cells (LSCs) drive relapse and chemoresistance through self-renewal, dormancy, and impaired differentiation. Resveratrol, a plant-derived polyphenol, has anti-leukemic potential, whereas its methylated analog, DMU-212, demonstrates enhanced efficacy, positioning it as a promising therapeutic candidate. This study investigated the anti-leukemic effects of DMU-212 on LSCs and its concomitant effects on hematopoietic stem cells (HSCs).

Materials and methods: The cytotoxic effects of resveratrol and DMU-212 on LSC (CD44+/CD133+/CD38-) and HSC (CD34+/CD38-) were assessed using CCK-8 assay. IC_{50}-induced apoptosis (AnnexinV/PI, DNA-fragmentation, active-caspase-3), anti-proliferation (CFSE), cell-cycle arrest (PI-staining) analyzed by flow-cytometry; cell survival (clonogenicity), gene expressions (qRT-PCR), genotoxicity (micronucleus), DNA damage (γH2AX) were evaluated.

Results: DMU-212 (IC_{50}:16.65 μM) exhibited higher cytotoxicity in LSCs than resveratrol (IC_{50}:95.43 μM). DMU-212 (16.65 μM) induced stronger late apoptosis, caspase-3 activation, and DNA fragmentation in LSCs relative to resveratrol (16.65 μM), accompanied by the regulation of apoptosis-related genes, including *BCL2L1, CASP3*, and *FASLG*. The survival fraction of DMU-212-treated LSCs reduced to 5.35% and the proliferation was significantly inhibited due to G_2/M arrest. The anti-proliferative effects were associated with the regulation of *CCNB1, CDC25A, TP53, ELK1, HRAS, JUN, KRAS, MAP2K2, JAK1, JAK2, SOCS3, STAT5B, AKT2, EIF4EBP, FOXO1, PDK1, PIK3CD*. DMU-212 also increased polyploidy, micronucleus formation, and γH2AX levels in LSCs. Both resveratrol and DMU-212 exhibited minimal adverse effects on HSCs.

Conclusion: DMU-212 selectively targets LSCs through apoptotic, anti-proliferative, and genotoxic mechanisms, while sparing healthy HSCs. These findings underscore its potential as a highly effective and low-toxicity therapeutic strategy for leukemia treatment.

Effects of resveratrol and DMU-212 on LSC and HSC (Fold change relative to control)

	Resveratrol (LSC)	DMU-212 (LSC)	Resveratrol (HSC)	DMU-212 (HSC)
Apoptosis	1.6	4.1	0.64	0.44
Caspase-3 Activation	2.8	4.2	0.39	1.25
DNA Fragmentation	3.2	8.6	0.2	1.25
Survival Fraction (%)	94.72	5.35	44.32	29
Proliferation Suppression	2	3.2	2.2	2.5
γH2AX	4.55	6.72	2.6	2.06

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P01.111.C Synergistic effect of Venetoclax and hypomethylating agents is independent of methylation loss

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Background: The BCL-2 inhibitor Venetoclax in combination with the hypomethylating agents (HMAs), 5-azacytidine (AZA) and decitabine (DEC) is clinically approved for use in acute myeloid

leukemia (AML). However, the molecular mechanisms underlying their synergy, particularly the role of DNA demethylation, remain poorly understood due to the dual effects of traditional HMAs, inducing both DNA demethylation and extensive DNA damage.

Material and methods: To investigate the role of DNA methylation in driving Venetoclax synergy with HMAs, we compared a novel DNMT1-specific inhibitor, which induces DNA demethylation with minimal DNA damage, to AZA and DEC. Cytarabine (ARAC), a DNA-damaging cytidine analogue without demethylating activity, was included as a comparator. AML cell lines were treated with each agent alone and in combination with Venetoclax, and synergistic effects were evaluated based on cell viability. Additionally, global methylation was assessed through enzymatic methyl-sequencing.

Results: DNA demethylation alone did not induce synergy with venetoclax. In contrast, a strong synergistic effect was observed when Venetoclax was combined with AZA and DEC, both of which induce DNA damage in addition to demethylation. Interestingly, ARAC, despite lacking demethylating properties, also demonstrated significant synergy with Venetoclax.

Conclusion: Preliminary results show that the synergy observed between Venetoclax and the traditional HMAs is primarily driven by DNA damage, not methylation loss. Interestingly, DNA demethylation alone, achieved by using the novel DNMT1-inhibitor, appear to be antagonistic, questioning the relevance of targeting DNA methylation. These findings suggest that the therapeutic benefit in AML is linked to DNA damage rather than changes in DNA methylation.

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P01.112.D Identification of a novel *PMS2* duplication in exons 1–12: implications in Lynch syndrome and hereditary cancer predisposition

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Background: Lynch syndrome (LS) is an autosomal dominant cancer predisposition syndrome caused by pathogenic variants in DNA mismatch repair (MMR) genes, including *PMS2*. While mutations in *PMS2* are well-documented, structural variations, such as large duplications, remain underexplored. Here, we report a novel duplication of *PMS2* exons 1–12 in two unrelated patients, highlighting its potential pathogenicity in LS.

Materials and methods: Two unrelated female cancer patients were referred for hereditary cancer predisposition investigation. The first, an 86-year-old with pancreatic and breast cancers, and the second, a 30-year-old with metastatic lung cancer and a family history of cancer. Genetic testing included Next Generation Sequencing (NGS) using Agilent SureSelect panel to exclude potential pathogenic mutations, and digital multiplex ligation-dependent probe amplification (MLPA) to assess copy number variations (CNVs) in cancer-related genes.

Results: A previously unreported duplication of exons 1–12 in *PMS2* gene was identified by digital MLPA and confirmed with conventional *PMS2*-specific MLPA. Subsequent family studies on the first patient's two sons revealed that one carried the same duplication.

Conclusion: This study identifies a novel *PMS2* exon 1–12 duplication possibly associated with LS. To our knowledge, this specific variant is absent from current literature. Its presence in two unrelated cancer patients along with familial segregation in one case, suggests a possible pathogenic role and supports its relevance in hereditary cancer predisposition. This finding expands the mutational spectrum of *PMS2* and highlights the importance of considering structural variations in hereditary cancer investigations. Further functional studies are needed to confirm its clinical implications in cancer.

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P01.113.A Genetic landscape of pediatric hematologic malignancies in Latvia: insights from whole-genome and RNA sequencing

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Background: Pediatric hematologic malignancies, such as B-cell acute lymphoblastic leukemia and acute myeloid leukemia, present distinct genetic profiles that are critical for risk stratification and treatment planning. This study aimed to explore the genetic landscape of these malignancies in a Latvian cohort using whole-genome sequencing and RNA sequencing.

Material and Methods: Whole-genome sequencing and RNA sequencing data were analyzed from 42 pediatric patients diagnosed with hematologic malignancies in Latvia between 2019 and 2022. Tumor and normal tissue samples were collected for genetic analysis, including structural variations and gene fusions.

Results: Somatic single nucleotide variants were identified in 130 target genes, with 28% of these genes exhibiting mutations in multiple patients. Frequently affected genes included *NRAS*, *MYC*, and *WT1*. Subtype-defining alterations such as hyperdiploidy and the *ETV6-RUNX1* fusion were observed among the most common findings in B-cell acute lymphoblastic leukemia. Chromosomal abnormalities were detected in 61% (25/41) of cases, with large chromosomal rearrangements (>30 kb) identified on chromosomes 7, 14, and X. Chromosome 21 was the most affected in terms of whole-chromosome copy number alterations. Notably, *ETV6-RUNX2* was discovered as a novel gene fusion in this cohort.

Conclusion: This study emphasizes the role of whole-genome sequencing in uncovering genetic complexities of pediatric hematologic malignancies, aiding in diagnosis and the development of targeted therapies.

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P01.114.B Molecular characterization of pediatric ALL: identifying key pathways and potential biomarkers through transcriptome analysis

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Background: Acute lymphoblastic leukaemia (ALL) represents 25% of pediatric malignancies, making it the most prevalent childhood cancer. This study aimed to characterize transcriptome profiles of B- and T-cell ALL to elucidate disease heterogeneity and identify potential novel biomarkers for personalized treatment approaches.

Material and methods: We performed RNA sequencing on pediatric bone marrow and peripheral blood samples from B-ALL and T-ALL patients. Comprehensive differential gene expression and pathway enrichment analyses were conducted to identify significant molecular signatures.

Results: Differential gene expression analysis revealed over 8,000 differentially expressed genes between B- and T-ALL. Immunoregulatory interactions between lymphoid and non-lymphoid cells emerged as potential contributors to T-ALL's poorer prognosis. In B-ALL, while pathway analysis revealed signatures distinguishing patients with and without relapse, the primary source of variability stemmed from distinct genetic subtypes. Further analysis indicated that increased oncogene expression and decreased tumor suppressor gene expression correlated with more aggressive disease in specific B-ALL subgroups and in T-ALL patients with poor outcome. Additionally, gene expression patterns showed poor correlation with immuno-phenotypes in T-ALL, consistent with existing literature.

Conclusion: This study provides valuable insights into ALL's molecular landscape, demonstrating distinct gene expression signatures between and within ALL subtypes. Our findings enhance understanding of biological mechanisms underlying ALL development and progression, particularly regarding the role of immunoregulatory pathways and the impact of dysregulated oncogene and tumor suppressor expression on disease severity. These insights emphasize the need for detailed molecular profiling to identify targeted therapeutic strategies and prognostic biomarkers, potentially advancing personalized medicine in pediatric ALL.

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P01.115.C Beyond BRCA: risk assessment and genetic aetiology of ovarian cancer in African women

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Background: Ovarian cancer, often termed the "silent killer," is hereditary in approximately 20% of cases. However, the genetic aetiology of ovarian cancer in African women remains poorly understood, with risk assessments based on studies in European populations and limited evidence of their applicability to African patients.

Material and methods: We conducted a scoping literature review of germline genetic testing in African women with ovarian cancer and evaluated the utility of risk assessment tools in 20 black African women diagnosed with ovarian cancer. These patients form part of a whole-exome sequencing project investigating ovarian cancer in African women.

Results: The review included 30 studies, predominantly involving African American and North African cohorts. Only four studies focused on ovarian cancer, while the rest involved breast cancer cohorts that included ovarian cancer cases. Across 110 African families with ovarian cancer, 75 disease-causing variants were identified, 92% of which were in *BRCA1* and *BRCA2*. Notably, 22 of the 30 studies exclusively tested for these genes, although variants in *BARD1*, *CHEK2*, *EP300*, *NTHL1*, *PALB2*, *PMS2*, and *TP53* were also reported.

Among the 20 patients recruited thus far risk assessment tools (Manchester, CanRisk, PREMM5) yielded low scores for 13 patients. The mean age of diagnosis was 48 years, and only six had a family history of cancer.

Conclusion: The findings highlight limited research on hereditary ovarian cancer in African women and the need for comprehensive approaches, such as exome sequencing, to refine genetic testing and risk assessment tools tailored to African women with ovarian cancer.

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P01.116.D PTPRJ and PTPRC activation as targets to counteract oncogenic FLT3-ITD-mediated cell transformation in AML

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Background: Nearly 30 % of Acute Myeloid Leukaemia (AML) patients carry activating mutations in the FMS-like tyrosine kinase 3 (FLT3). Most common are Internal Tandem Duplication (ITD) mutations in the juxtamembrane domain of this receptor tyrosine kinase, causing loss of auto-inhibition of the kinase resulting in constitutive FLT3 activity, retention of the immature receptor in the biogenesis route and aberrant activation of STAT5 signalling. Thus, FLT3-ITD proteins induce uncontrolled cell proliferation, block cell differentiation and drive cell transformation. Receptor Protein Tyrosine Phosphatases (RPTP) antagonize FLT3 activities. Here we address the effects of PTPRJ and PTPRC overexpression on FLT3 phosphorylation and FLT3 downstream signalling.

Material and methods: FLT3-WT/ITD and PTPRJ/PTPRC proteins were co-expressed using transient transfection in HEK293T cells. Cell lysates were processed with immune blotting and FLT3 phosphorylation as well as activation of downstream targets AKT, ERK1/2 and STAT5 were characterized.

Results: Overexpression of both RPTP dephosphorylated FLT3 wild type and consequently diminished phosphorylation of downstream signalling proteins AKT and ERK1/2. While PTPRC efficiently dephosphorylated FLT3-ITD resulting in increased maturation of the receptor and significant reduction of downstream signalling, PTPRJ hardly affected FLT3-ITD activity. This difference can be explained by FLT3-ITD-mediated ROS

production resulting in PTPRJ oxidation, which lead to diminished phosphatase activity.

Conclusion: Activation of RPTP, in particular PTPRC, is an attractive target to antagonise oncogenic FLT3-ITD signalling. In particular, abrogation of RPTP oligomerization, which subsequently increases their activity, is an attractive way to counteract FLT3-ITD-mediated cell transformation and therefore target AML.

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P01.117.A FIRE: a decision tree algorithm for automating oncogenicity classification of cancer somatic variants using the ClinGen/CGC/VICC framework

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Background: Accurate classification of somatic variants is critical for precision oncology. The ClinGen/CGC/VICC 5-tier framework provides structured guidelines to support clinical interpretation. However, no tool fully integrates this framework into an automated system. ClinVar annotates a subset of somatic variants, VIC employs a hybrid approach with FDA-approved evidence, and OncoKB prioritizes therapy-linked predictions over oncogenicity classification. The lack of a standardized system limits consistency in variant interpretation and clinical decision-making. To address this, we developed FIRE, a decision tree-based model to automate classification, enhance reproducibility, and minimize variability.

Material and methods: In this preliminary study, FIRE was tested on 253 benchmark variants (oncogenic and benign) from five cancer genes in ClinVar. The model applies weighted scoring to ClinGen/CGC/VICC evidence categories, integrating population data, computational predictions, and variant type. FIRE's performance was assessed using F1-score, sensitivity, and false positive rate (FPR). It was validated on 157 SNVs and Indels in *TET2* from colorectal (CRC) and ovarian cancer (OC) AACR GENIE datasets.

Results: FIRE correctly classified 70% of benchmark variants, achieving an F1-score of 0.92, sensitivity of 1, and FPR of 0.58. In CRC and OC datasets, FIRE classified 44% of previously unclassified *TET2* variants, identifying 30 oncogenic, 37 likely oncogenic, and 2 likely benign variants. The remaining 56% were Variants of Uncertain Significance.

Conclusion: These findings highlight FIRE's ability to classify somatic variants, particularly likely oncogenic or oncogenic. Future work will benchmark FIRE against existing tools, refine feature weighting, integrate additional ClinGen/CGC/VICC evidence, and expand classification to more cancer genes.

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P01.118.B Melanogenesis-related polymorphisms association with melanoma and carcinoma development and aggressiveness

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Background: Malignant melanoma is a rapidly progressing cancer responsible for 80% of skin cancer deaths, despite being only 4% of skin cancer cases. In contrast, basal cell carcinoma is the most common skin disorder worldwide. This study investigates the relationship between two genetic polymorphisms related to melanogenesis: rs1126809 in the *TYR* gene and rs2733832 in the *TYRP1* gene, and their association with melanoma and carcinoma.

Material and methods: Samples of blood, waxed tissue, or saliva were collected from 155 healthy controls, 127 melanoma patients, and 112 carcinoma patients. DNA was extracted, and the genes of interest were genotyped using TaqMan probes. The results were analyzed using a Pearson chi-square test. A meta-analysis was also conducted, incorporating published studies related to our single nucleotide polymorphisms (SNPs) and the diseases under investigation, which significantly increased the sample size.

Results: In our cohort, no significant differences were observed; however, there was a tendency to develop melanoma more frequently in individuals with the A risk allele for the rs1126809 (TYR) polymorphism, following a dominant model (p-value: 0.084). After performing the meta-analysis, we found a strong association between rs2733832 (TYRP1) and melanoma (p-value: 6.21*10^-6), as well as between rs1126809 (TYR) and melanoma (p-value: 2.72*10^-7), and a trend toward association with carcinoma (p-value: 0.056).

Conclusion: Identifying reliable biomarkers for melanoma is vital due to its high mortality rate. This study suggests that SNPs rs1126809 and rs2733832 may serve as decision-support biomarkers for managing patients at risk for melanoma.

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P01.119.C Germline pathogenic variants in patients with a suspicion of hereditary paraganglioma-pheochromocytoma syndrome-a single centre experience

Fulvia Brugnoletti, *Aurélie Ayme, Amandine Cadènes, Jean-Louis Blouin, Jean-Christophe Tille, Claudio De Vito, Frédéric Triponez, Maria Mavromati, Sophie Leboulleux, Pierre Olivier Chappuis*

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Background: Around 40% of patients diagnosed with paraganglioma (PGL) and/or pheochromocytoma (PCC) carry germline pathogenic/likely pathogenic variants (PV/LPV) in one susceptibility genes, mainly in succinate dehydrogenase genes (SDHx). Individuals harboring germline SDHx PV/LPV tend to develop tumours at a younger age with multifocal and/or bilateral disease, high malignant incidence, and recurrence rate.

Material and methods: We reviewed all cases addressed to our Oncogenetic Unit for a suspicion of hereditary PGL/PCC syndrome from 2016 to 2024. Germline analysis was performed using the following gene panel: *FH, MAX, MEN1, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127* and *VHL*.

Results: 28 patients underwent genetic testing for a suspicion of hereditary PGL/PCC syndrome. In 9/28 (32%) a germline PV/LPV was identified in the following genes: *SDHB* (5/9), *SDHA* (1/9), *SDHAF2* (1/

9), *SDHD* (1/9), and 1 *VHL* (1/9). Among these cases, 5 presented with single (n = 2) or multiple (n = 3) PGLs, 1 with PCC, while 2 had a diagnosis of early onset renal cancer. One patient was referred for a single cervical PGL identified during baseline assessment for a gastric GIST. Median age at diagnosis was 34 years (range, 12–53). Immunohistochemical data were available for 7/9 tumoral tissues: all of them showed a loss of SDH expression.

Conclusion: Around 30% of patients with a suspicion of PGL/PCC hereditary syndrome harbor germline PV/LPV in SDHx genes, with the highest proportion in *SDHB*. These results highlight the importance of germline evaluation for all patients with features of hereditary PGL/PPC syndrome.

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P01.120.D Clinical and molecular spectrum of DDX41 variants in Korean patients with hematologic malignancies

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Background: Hereditary hematologic malignancy is characterized by an increased risk for hematologic malignancies due to germline variants in cancer predisposition genes. Among them, germline *DDX41* variants are the most frequent especially in adults and found in 1.5% of myeloid neoplasms. In this study, we investigated the frequency and significance of *DDX41* variants in Korean patients.

Material and methods: We retrospectively reviewed 713 patients who were suspected as hematologic malignancies and underwent targeted sequencing of 50 genes in our institution from August 2020 to July 2023. Among them, we analyzed the clinical characteristics of patients with *DDX41* variants.

Results: Of 713 patients, 29 patients had germline and/or somatic *DDX41* variants and a total of 22 variants were identified. Fifteen variants were presumable germline variants, and 11 patients (1.5%) were diagnosed as hematologic neoplasms with germline *DDX41* variant presenting as 6 MDS (6/90, 6.7%), 4 AML (4/165, 2.4%), and 1 MPN (1/148, 0.7%). The median age of hematologic neoplasms with germline *DDX41* variant was 69 with male predominance (8:3). *DDX41* Y259C variant was the most common germline variant (63.6%) and the R525H variant was the most common somatic variant (24.1%).

Conclusion: Our study demonstrated the clinicopathological characteristics of *DDX41* variants in Korean patients with hematologic malignancies. Our findings highlight the importance of assessment of *DDX41* variants in hematologic malignancies.

C.O.I. Disclosure:

B. Kim: None.

P01.122.B Longitudinal DNA-methylation profiling of CLL patients with simple and complex karyotypes

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Background: Chronic lymphocytic leukemia (CLL) is characterized by theaccumulation of malignant B cells with clinically heterogeneous outcomes.Aberrant global DNA hypomethylation and promotor specific hypermethylation attumor suppressor genes are a common feature during tumor progression. DNAmethylation signatures also distinguish CLL into subtypes with distinctprognostic outcomes. CLL patients with complex karyotypes show an overall poorprognosis but the underlying mechanism remains unclear. The aim of this studyis to compare CLL patients with normal and complex karyotypes and to gaininsight into the role of DNA methylation in tumor progression mechanisms and touncover potential new treatment options.

Materials and methods: We analyzed DNA methylation profiles from 15 patients withTp53 WT and either normal or complex karyotypes at diagnosis and relapse. DNAmethylation profiles were obtained by targeted enriched bisulfite sequencingusing an 84 MB bait library targeting CpG islands and promoter regions.Methylation profiles were called using bismark 0.24.2 using the latest hs1genome as the reference.

Results: Initial data revealed differences in global DNA methylationprofiles between baseline CLL and relapse, with only few regionshypermethylated and most regions hypomethylated upon relapse. Comparison between a favorable andunfavorable karyotype will be presented upon the conference.

Conclusion: The methylation profiles between basic and relapsed CLLreveal only few differences, which might be attenuated by the complexity of thepatient's karyotype. Further studies will show the interconnection between DNAmethylation alterations and disease progression.

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P01.123.C Genetic landscape of Lynch syndrome:insights from a Turkish cohort

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Background: Lynch syndrome (LS) is an autosomal dominant genetic condition associated with a significantly increased risk of colorectal, endometrial, and various other cancers. The syndrome arises due to mutations in DNA mismatch repair (MMR) genes, most commonly MLH1, MSH2, MSH6, EPCAM and PMS2. These mutations lead to microsatellite instability, which promotes tumor formation. Lynch syndrome accounts for approximately 3–5% of colorectal cancers, making it one of the most common hereditary cancer syndromes.

Material and methods: A total of 131 patients who were referred to the Department of Medical Genetics at Ege University Hospital between 2018 and 2024 and were found to have variants in Lynch syndrome-associated genes in a cancer panel were analyzed.

Results: Among the 131 patients analyzed, *MLH1* variants were detected in 49 patients, *MSH2* in 25, *MSH6* in 34, and *PMS2* in 26. *MLH1*, *MSH2*, and *PMS2* variants were most commonly found in colorectal cancer patients (20, 11, 7 cases, respectively), while *MSH6* variants were most frequent in breast cancer patients(16 cases). A total of 15 variants were classified as pathogenic, and 10 as likely pathogenic. Copy number variations (CNVs) were detected in 19 patients, including 10 deletions and 9 duplications.

Conclusion: These findings highlight the role of *MLH1, MSH2, MSH6*, and *PMS2* variants in cancer susceptibility and underscore

the importance of genetic analysis in identifying individuals at risk. Additionally, this study contributes to the literature by shedding light on Lynch syndrome-associated genetic variants in the Turkish population.

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P01.124.D Transcriptomic profiling of cancer-associated fibroblasts: identifying key biomarkers and therapeutic targets for targeted therapy

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Background: The characteristics of a tumor are largely determined by its interaction with the surrounding micro-environment (TME). TME consists of both cellular and non-cellular components. Cancer-associated fibroblasts (CAFs) are a major component of the TME. They are a source of many secreted factors that influence the survival and progression of tumors as well as their response to drugs. Identification of markers either overexpressed in CAFs or unique to CAFs would pave the way for novel therapeutic strategies that in combination with conventional chemotherapy are likely to have better patient outcome.

Material and methods: Fibroblasts have been derived from Benign Prostatic Hyperplasia (BPH) and prostate cancer. RNA from these has been used to perform a transcriptome analysis in order to get a comparative profile of normal and cancer-associated fibroblasts.

Results: The study has identified 818 differentially expressed mRNAs and 17 lincRNAs between normal and cancer-associated fibroblasts. Also, 15 potential lincRNA-miRNA-mRNA combinations have been identified which may be potential biomarkers.

Conclusion: This study identified differentially expressed markers between normal and cancer-associated fibroblasts that would help in targeted therapy against CAFs/derived factors, in combination with conventional therapy. However, this would in future need more experimental validation

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P01.126.B BRCA1/2 population genetic screening: challenges and benefits

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Background: Approximately 80% of BRCA1/2 positive breast and ovarian cancers are diagnosed at stages II-IV. Despite modern treatments, morbidity, mortality, and associated costs remain significant. Early identification of BRCA1/2 carriers is critical to improving outcomes for individuals at risk of HBOC. This pilot study evaluates the feasibility of implementing a BRCA1/2 population screening program in Latvia.

Material and methods: This pilot study was conducted using the digital engagement and consent management tool "Longenesis Engage". The invitations were sent by e-mail. Eligible participants (age 25–59, without personal cancer history) who provided informed consent were included in the pilot. Saliva samples were collected and tested for BRCA1/2 SNV and CNV. Negative results were communicated digitally, while individuals with pathogenic variants were contacted personally, referred to medical geneticists, and enrolled in the surveillance program.

Results: In total, 3,438 invitation emails were sent. Of these, 49% were open, and 54% of recipients who opened the email visited the digital platform for project details. Among platform visitors, 79% provided informed consent, primarily via digital signature. Saliva samples were collected by 67% (490), yielding an overall response rate of 14.26% for this initial phase. Additionally, three gynecological practices directly invited women to participate, with a positive response from 49 women. Among the 539 analyzed cases, BRCA1/2 pathogenic variants were detected in 8, resulting in a detection rate of 1.49%.

Conclusion: The study demonstrates that BRCA1/2 population screening via digital communication channels is feasible. Findings confirm a high BRCA1/2 pathogenic variants frequency in Latvia, although screening methodology requires further refinement.

C.O.I. Disclosure:

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P01.127.C Molecular variant detection in non-small cell lung cancer- internal validation and external quality control

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Background: To optimize treatment for non-small cell lung cancer (NSCLC), accurate molecular characterization of tumors is essential. We validated an RNA-based NGS assay for detecting clinically significant genetic variants in NSCLC tissue samples.

Material and methods: To validate the Archer Fusion Plex Lung (AFPL) assay, 1190 NSCLC specimens were analyzed from 2023–2024. AFPL results were verified using conventional IVD tests like qPCR, FISH, and a DNA-based NGS assay. A minimum of 0.5 million reads per sample and an Internal Sample Quality Control index ≥10 were required. Seraseq FFPE Tumor Fusion RNA Reference Material was used for fusion detection control. The laboratory participated in the EMQN external quality control program (Lung Cancer: Common Biomarkers, New and Emerging Biomarkers; Oncogene Panel; and Fusions) to assess performance.

Results: Valid molecular results were obtained for 1024 (86.1%) samples; 13.9% failed NGS or data analysis quality control. Actionable variants were detected in 581 (56.7%) of valid samples. RNA concentrations ≥10 ng and PreSeq Cp values <29 defined acceptable thresholds. Validation established allele frequency reporting thresholds of ≥4.9% (≥10 altered reads at ≥100x coverage) for SNVs and ≥10% fusion site reads (≥5 supporting reads from ≥3 origins) for fusions, showing strong concordance with FISH and qPCR. Median values for Total Fragments, RNA QC, and RNA fragment length were 1,255,393; 206, and 124, respectively. All completed EMQN schemes yielded satisfactory results; the Fusion scheme is under evaluation.

Conclusion: RNA-based NGS provides a robust diagnostic solution for precise identification of therapeutically relevant variants across multiple genes in NSCLC. **Grants**:IGICHP_3.3/22

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P01.128.D Prevalence of *RNF43* variants in patients undergoing genetic testing for polyposis-associated genes

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Background: Germline pathogenic variants in *RNF43*, a negative regulator of the Wnt signaling pathway, are associated with predisposition to serrated polyposis syndrome and colorectal cancer. This gene has been recently incorporated into the diagnostic routine of patients with suspected familial polyposis. In this study, we analyzed the prevalence and distribution of *RNF43* variants in patients with a suspicion of genetic predisposition to polyposis over the past four years, and we explored the occurrence of *RNF43* variants in individuals with other hereditary cancer suspicions.

Material and methods: The *RNF43* gene was retrospectively analyzed in 6278 patients who underwent genetic testing based on personal or family history suggestive of hereditary cancer. Patients were grouped based on the primary phenotype-related genes requested for analysis: group A comprised patients with clinical indication of gastrointestinal polyposis and/or related cancer and group B the rest of individuals with hereditary cancer suspicion.

Results: No clinically actionable variants in *RNF43* were found in either group. *RNF43* VUS were identified in 1.33% (17/1282) of patients in group A. In group B, 0,58% (29/4996) of patients also carried *RNF43* VUS.

Conclusion: Although *RNF43* variants were more frequent in group A, their overall prevalence remains low and no clear association with polyposis was observed. Analysis considering the presence of different histologic polyp subtypes and functional evaluation of the identified VUS is required to obtain stronger evidence on the utility of the inclusion of *RNF43* in routine genetic testing.

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P01.129.A Mutational and expression profiling of *TERT* and *CTNNB1* in hepatocellular carcinoma, cirrhotic, and non-cirrhotic liver tissues from Brazilian patients

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Background: Hepatocellular carcinoma (HCC) is a major global health challenge with poor prognosis in advanced stages. It frequently develops in cirrhotic livers due to specific risk factors and involves genetic mutations. *TERT* and *CTNNB1* somatic mutations are prevalent in HCC but remain underexplored in Brazilian patients. This study investigates the association of *TERT* promoter mutations (-146C>T, -124C>T) and *CTNNB1* exon 3 mutations with HCC in Brazil.

Material and methods: Mutation analysis was performed on 85 liver tissue samples, including 26 tumor/surrounding tissue pairs and 33 unpaired samples (16 HCC, 9 cirrhotic, and 8 non-cirrhotic). DNA from cryopreserved or formalin-fixed tissues was analyzed by Sanger sequencing, and RNA from cryopreserved tissues was used for *TERT* expression analysis via RT-qPCR.

Results: The -146C>T mutation was rare in HCC (2.4%) and absent in cirrhotic and non-cirrhotic tissues. The -124C>T mutation was more frequent in HCC (45.2%) and cirrhotic tissues (19%) than non-cirrhotic tissues (0%), showing significant differences (HCC vs. non-cirrhotic, $P < 0.0001$; cirrhotic vs. non-cirrhotic, $P = 0.0485$). In paired samples, -124C>T was exclusive to HCC in 46.2% of cases. *CTNNB1* mutations were more common in HCC (17,24%) than cirrhotic (5,5%) and non-cirrhotic tissues (0%) and correlated with -124C>T ($P = 0.04$). Multivariate analysis identified -124C>T as a strong HCC predictor (HR = 10.23, 95% CI 2.18–48.1, $P = 0.003$). *TERT* expression was significantly higher in tumoral tissues ($P < 0.0001$) but not linked to promoter mutations.

Conclusion: *TERT* promoter -124C>T and *CTNNB1* mutations are promising biomarkers for HCC in Brazilian patients, aiding early detection, risk assessment, and potential therapeutic development.

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P01.130.B Investigation of early onset breast cancer in West Africa

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Background: Early-onset breast cancer (EOBC) is a global public health concern, with a disproportionate burden in sub-Saharan Africa. Women in this region often face late-stage diagnosis and poor survival rates, with mortality reaching 80%. The genetic contributions to EOBC in African populations are understudied, limiting opportunities for early detection and personalized care.

Materials and methods: This pilot study uses whole genome sequencing (WGS) to investigate the genetic basis of EOBC in 100 Senegalese women diagnosed at ≤30 years. Phase 1 focuses on identifying pathogenic variants in known predisposition genes (BRCA1/2, ATM, PALB2, CHEK2, RAD51C, RAD51D). Phase 2 explores novel genetic risk factors potentially unique to African populations. The study integrates capacity-building efforts, including training in genomic data analysis, sample processing, and cascade testing.

Results: The cohort includes 100 women aged 15–30 years at diagnosis. Preliminary findings reveal that 50% of cancers are triple-negative (ER/PR/HER2-). Key data on stage, treatment modality, BMI, parity, menarche, family history, medication exposure, and contraceptive use are being analyzed. Initial results identify pathogenic variants in predisposition genes and suggest potential novel variants unique to the cohort. Stakeholder engagement has begun to enhance awareness of genetic testing and personalized oncology practices.

Conclusion: This study aims to advance understanding of EOBC's genetic basis in Senegalese women and establish a genomic medicine framework. The findings will inform strategies to improve early detection, guide treatments, and reduce disparities in breast cancer outcomes across Africa.

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P01.131.C Detection and characterization of BCR-ABL1 transcripts in acute and chronic leukemia: the value of molecular diagnosis and multiplex approach

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Background: Acute and chronic leukemias are hematological malignancies associated with the translocation t(9;22)(q34;q11), which is responsible for the formation of the BCR-ABL1 fusion transcript. In chronic myeloid leukemia (CML), the majority of transcripts are e13a2 and e14a2, while in acute lymphoblastic leukemia (ALL), the predominant forms are e1a2 and, on occasion, rare variants. Precise identification of the BCR-ABL1 transcript is imperative for diagnosis, therapeutic follow-up and adaptation of treatment with tyrosine kinase inhibitors (TKIs).

Material and methods: A study was conducted on a series of 127 patients with CML and AL at the Medical Genetics and Molecular Biology Laboratory of Hassan II University Hospital in Fez. The diagnostic workflow included the following techniques: Karyotype, Fluorescence in situ hybridization (FISH), Standard RT-PCR and the Multiplex RT-PCR.

Results: the majority of CML patients exhibited the typical e13a2 and e14a2 transcripts, while the majority of ALL cases expressed e1a2. However, multiplex RT-PCR allowed the detection of rare BCR-ABL1 transcript variants that were not identified by standard RT-PCR like the transcripts e19a3 and e8a2. The principal advantage of multiplex RT-PCR lies in its capacity to detect multiple transcript variants simultaneously, thus reducing the incidence of diagnostic errors that could lead to misclassification of patients and delays in the administration of appropriate treatment.

Conclusion: This study underscores the significance of multiplex RT-PCR in the diagnosis of acute and chronic leukemias associated with BCR-ABL1. By enhancing the detection of rare transcripts and reducing false-negative results, this approach improves diagnostic accuracy and contributes to enhanced patient management.

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M. Jalte: None.

P01.132.D MINAS involving *BRCA1*: description of two patients and considerations for genetic counseling and risk assessment

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Background: Multilocus Inherited Neoplasia Allele Syndromes (MINAS) are an emerging concept in oncogenetics, referring to individuals carrying multiple pathogenic variants in cancer susceptibility genes (CSGs). With the rise of NGS, these patients are increasingly identified, but the impact on risk assessment remains poorly understood.

Material and methods: NGS panel analysis of CSGs was performed in two unrelated patients. MLPA was used for confirmation of an identified *MSH2* deletion.

Results: The first patient, a 46-year-old woman with breast cancer, was found to carry a pathogenic *BRCA1* variant (c.3285del,p.(Lys1095AsnfsTer14)) and a pathogenic *MSH2* variant c.(428_576)_(577_864)del, inherited from her father and mother, respectively. Family history revealed breast cancer and melanoma on the paternal side and Lynch syndrome-associated tumors on the maternal side. The second patient had two primary brain tumors (pilocytic astrocytoma at age 2 years, pleomorphic xanthoastrocytoma at age 18 years) and a family history of thyroid cancer (father), ovarian cancer (mother, deceased at 52), and ovarian, breast, and spleen cancer (maternal grandmother). Genetic testing identified a pathogenic *BRCA1* variant (c.5266dup,p.(Gln1756ProfsTer74)) and a likely pathogenic *CHEK2* variant (c.470T>C,p.(Ile157Thr)) associated with low penetrance.

Conclusion: These cases expand the knowledge on *BRCA1*-related MINAS, emphasizing the need for better risk assessment in carriers of multiple CSG variants, within this rare context. The *BRCA1-CHEK2* combination is very uncommon and may contribute to a broader tumor spectrum, despite neither gene being linked to increased brain tumor risk. Further investigation is needed to clarify potential modifying factors influencing tumor development in similar cases.

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P01.133.A The effect of miR30c-2-3p on METTL14 and its role in migration inhibition in breast cancer

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Background: High expression of miR30c-2-3p is linked to good prognosis in breast cancer (BC). Analysis using the GEPIA2 database revealed increased METTL14(methyltransferases) expression in BC compared to healthy tissues. Considering the regulatory effects of miRNAs on m6A modifications, this study aimed to investigate miR30c-2-3p's role in regulating m6A modifications, its impact on migration, and its relationship with BC.

Material and methods: MCF12A (healthy) and MCF7 (BC) cells were transfected with miR30c-2-3p mimics. miR30c-2-3p and METTL14 RNA levels were measured by RT-PCR, while protein levels were assessed via western blot (WB). Migration was evaluated using scratch assays, and m6A levels were determined through a colorimetric method.

Results: Transfection with miRNA mimics significantly increased miR30c-2-3p expression in MCF7 and MCF12A cells ($p < 0.01$). In MCF7 cells, METTL14 expression decreased by 39.4%, 50.9% at the RNA level and 79%, 56.2% at the protein level after 24 and 48 hours, respectively ($p < 0.001$). m6A levels reduced by 28.9%, 46.2%, and migration decreased by 30%, 44%, respectively. In MCF12A cells, METTL14 expression decreased by 25.6%, 30.9% at the RNA level and 40.9%, 43.2% at the protein level at 24 and 48 hours, respectively ($p < 0.001$). m6A levels reduced by 83.7%, 63.9%, and migration inhibited by 66%, 72%, respectively.

Conclusion: miR30c-2-3p overexpression reduced METTL14 and m6A levels while inhibiting migration. Although migration results decreased in both cell lines, MCF7 demonstrated less migration compared to MCF12A, likely due to the activation of alternative pathways. These findings highlight miR30c-2-3p's role in BC biology and its potential as a therapeutic target.

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P01.134.B Exploring the role of EMT genes and its regulatory microRNAs in prostate cancer risk susceptibility

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Background: Epithelial-mesenchymal transition (EMT) is a crucial step in PCa invasion and metastasis. EMT is tightly regulated by multiple factors, including several miRNA. MiRNAs are essential for controlling the survival, growth, and metastasis of cells. The present study has been designed to investigate the expression and polymorphic analysis of EMT markers in PCa & CRPC and predict their targetome.

Material and methods: We have analyzed the gene expression by RT-PCR in PCa ($n = 50$), CRPC ($n = 50$), and control subjects ($n = 50$) serum and in PCa ($n = 50$) and control ($n = 50$) tissue sample. The polymorphism was investigated in serum sample by PCR-RFLP. The targetome of EMT genes was analyzed using bioinformatic tools. MiRNA profiling was carried out in both serum and tissue samples.

Results: Our results indicated significantly elevated expression of SNAIL, ZEB2, and VIM in PCa tissue and serum. Similarly, they were reported to be up regulated in CRPC serum. Moreover, a significant association was also established between VIM (rs11254434) C/T, SNAIL (rs1543442)A/C, and ZEB2 (rs13389578)T/C polymorphism with increased PCa risk. The targetome analysis reveals that EMT genes were regulated by miRNA 130b, 21, & 375 that involved in the PCa progression. The expression profiling of all miRNA were found to significantly upregulated in both serum and tissue samples.

Conclusion: Collectively these results revealed a direct link among EMT marker genes with miRNA 130b, 21, & 375 which were significantly linked to increased risk of PCa. Thus, EMT genes and its related miRNA can be exploited as a potential therapeutic target in PCa.

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P01.135.C Consumer acceptability about extending the role of the victorian cancer registry into the germline genetic testing pathway

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Background: In the era of precision public health, population-based cancer registries could become timely data sources to identify individuals who would benefit from germline genetic testing. To do so, may require registries to contact individuals. Assessing consumer acceptability of new service models is critical to successful implementation and delivering person-centred, value-based healthcare. We sought to examine consumer acceptability of extending the Victorian Cancer Registry into the germline genetic testing pathway.

Material and methods: Through the Victorian Cancer Registry, individuals recently diagnosed with a suspected pathogenic cancer or tumour were identified and invited to participate in a 1:1 or group interview. The interview schedule and inductive and deductive content analysis approaches were informed by the Theoretical Framework of Acceptability which covers seven constructs of acceptability: affective attitude, ethicality, perceived effectiveness, burden, intervention coherence, opportunity costs, & self-efficacy.

Results: Nine interviews with a total of 19 participants were conducted. Participants were supportive of extending the role of the registry, as utilising health data aligned with their personal values, and was perceived to effectively improve patient care. Data security, verification of the legitimacy of the registry, consideration for the timing of the contact, and suggestions for ways to reduce the emotional and mental burden on the consumer around the time of diagnosis, were discussed.

Conclusion: Optimising cancer registries to improve clinical genomics was acceptable to consumers, as it was timely, easy to understand and action, if legitimacy and data security were assured.

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P01.136.D The genetic landscape of hereditary cancer syndromes in Romania: a 4 year perspective

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Background: Germline pathogenic or likely pathogenic variants in well-established cancer predisposition genes contribute to hereditary cancer syndromes such as HBOC, Lynch, and Li-Fraumeni, posing a significant burden on patients and healthcare systems. The genetic landscape of hereditary cancers remains underexplored in the Romanian population.

Material and methods: Between 2020 and 2024, 1,359 Romanian patients underwent hereditary cancer genetic testing at Genekor S.A. using a 52-gene next-generation sequencing (NGS) panel. Clinical data included oncologic diagnosis, age at diagnosis, and family history. Variants of uncertain significance (VUS) were excluded from analysis.

Results: Among the tested patients, 319 (23.5%) carried pathogenic or likely pathogenic variants. Single-nucleotide polymorphisms (SNPs) were identified in 309 individuals, while 10 had copy number variations (CNVs). BRCA1 was the most frequently affected gene, followed by CHEK2 and BRCA2, together accounting for 49.1% of positive results. Lynch syndrome-related variants (MLH1, MSH2, MSH6, PMS2, and EPCAM deletions) were found in 11% of cases, while MUTYH variants were present in 6.9%

Conclusion: This study provides a comprehensive analysis of pathogenic variants associated with hereditary cancer syndromes in Romania. Findings are correlated with personal and familial cancer history, detailing the most frequent variants and variant types (missense, nonsense, frameshift) across different genes. These insights contribute to a broader understanding of hereditary cancers in Romania.

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P01.137.A Germ-line pathogenic gene variants among pancreatic cancer patients

Zuzanna Frydrych, Malgorzata Lisik, Anna Fiszer-Kierzkowska, Magdalena Kalinowska-Herok, Magdalena Mazur, Aleksandra Pfeifer, Marta Cieslicka, Agnieszka Pawlaczek, Wieslaw Bal, ***Malgorzata Oczko-Wojciechowska***

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Background: Introduction: Pancreatic cancer is an aggressive tumor with a very high mortality rate. Genetic factors also contribute to an increased risk of pancreatic cancer. It is now considered that up to 20% of people diagnosed with pancreatic cancer have the disease linked to an inherited predisposition. Identifying germline variants makes it possible to assess the risk of an inherited form of the disease in the patient's relatives and qualify for molecularly targeted therapies. The study aimed to assess the frequency of BRCA1/2, PALB2, TP53 gene variants and mismatch repair (MMR) genes associated with Lynch syndrome in patients diagnosed with pancreatic cancer.

Methods and material: Between 2021 and 2024, 81 patients diagnosed with pancreatic adenocarcinoma received genetic testing at our genetic clinic, using Next Generation Sequencing using Illumina MiniSeq.

Results: Germline pathogenic variants were detected in 11% of patients, of which 7% of all detected variants were alterations in the BRCA1 and 2 genes. Other detected variants were in the TP53 gene (c.309C > G), MSH2 gene c.902_903del and MSH6 gene c.3226C > T.

Conclusions. The association with hereditary predisposition was demonstrated in more than 11% of pancreatic cancer patients. The study confirmed the most frequent occurrence of pathogenic variants in BRCA1/2 genes in patients with pancreatic cancer. In addition, it showed that pancreatic cancer may also be associated with variants specific for Lynch and Li Fraumeni syndrome. Therefore, genetic testing should be considered for any patient diagnosed with pancreatic cancer.

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P01.138.B LUCID: a French multicentric prospective study to assess the risk of DDX41-related hematologic malignancies

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Background: *DDX41* Germline pathogenic or likely pathogenic variants (PV/LPV) are found in about 5–6% of patients with acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) or idiopathic cytopenias. Unlike major germline cancer predispositions, diagnosis of hematologic malignancy for carriers is not made at an early age (median ranging from 65 to 69 years). As most reports on *DDX41* gene refer to case series of hematology patients, the risk of developing hematologic disorders in carriers is not precisely known. Thus, the attitude towards germline testing, and follow-up measures for related carriers, are not homogenous and further prospective data are needed to establish guidelines.

Material and methods: LUCID (LeUkemia in Carriers of germline *DDX41* mutation) is a French family-based multicentric cohort study designed to assess the cumulative risk of hematologic malignancies according to the age of *DDX41* PV/LPV carriers. Index cases (with clonal hematopoietic stem cell neoplasms and known or suspected to carry a *DDX41* PV/LPV) are recruited during genetics consultations (health questionnaire +/− saliva sampling). Their first, second and third degree relatives are then enlisted by

mail (questionnaire, saliva sample and CBC). A total of 910 participants are expected for a total study duration of 74 months.

Results: Here, we describe the first 14 months of the feasibility phase of the LUCID project, gathering the inclusion of more than 150 participants (≥30 families).

Conclusion: The inclusion protocol is up and running, and the LUCID study could therefore be extended by opening it up to collaboration with more investigation centers, including international centers.

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P01.139.C Discovering shared gene mutations across different tumor types using Liquid biopsy

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Background: Liquid biopsy is a minimally invasive approach for profiling tumor genetics through circulating tumor DNA (ctDNA). This study aimed to genotype diverse tumor types using a targeted gene panel, uncovering mutations across cancer types to evaluate their relevance in precision oncology.

Material and methods: The study enrolled 40 oncology patients: 19 with colorectal carcinoma (CRC), 12 with NSCLC, and 9 with breast cancer. ctDNA was extracted from plasma samples and subjected to targeted sequencing of 484 cancer-related genes. Bioinformatics analyses were performed to identify and interpret somatic mutations.

Results: Mutations in BRCA1 (c.1016del) were identified in 58.3% of NSCLC and 21.1% of CRC patients, as well as in breast cancer, where its role is well-documented. BRCA2 (c.9097dup) was detected in 50% of NSCLC and 47.4% of CRC cases. Mutations in GATA3 (c.404del), typically associated with breast cancer, were found in 16.7% of NSCLC and 15.8% of CRC cases. Notably, KMT2D (c.2533del), known for its role in epigenetic regulation, appeared in 26.3% of CRC, 50% of NSCLC, and 11.1% of breast cancers. PAX5 (c.963del), linked to hematologic malignancies, was unexpectedly prevalent across solid tumors (CRC: 73.7%; NSCLC: 83.3%; breast: 11.1%). Mutations in HNF1A (c.863_864insC and c.864del), commonly associated with liver cancer, were present in all three tumor types.

Conclusion: Cross-cancer mutations suggest that tumor classification may evolve from location-based to mutation-driven paradigms, enhancing precision oncology strategies.

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P01.140.D Low diagnostic yield in gene panel testing of Finnish melanoma patients

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Background: Some melanomas are attributed to hereditary predisposition, with *CDKN2A* variants being the most significant. In other populations, diagnostic yield of gene panel testing has been 5–10%.

Material and methods: This study assessed the diagnostic yield of melanoma gene panel testing in 72 patients diagnosed with melanoma, having either multiple melanoma (≥3) and/or family members with melanoma and/or pancreatic cancer referred to Clinical Genetics Unit at the university hospital in Helsinki, Finland during 3/2019-11/2023. We also analyzed the prevalence of potentially pathogenic variants in *CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP, TERT, MITF*, and *MC1R* genes in Finnish and European ancestry group in gnomAD v4.1 database.

Results: Gene panel testing was outsourced to multiple laboratories with varying gene content. The most significant genes, *CDKN2A, CDK4, BAP1*, and *POT1* were included in all or almost all panels. The median age at the diagnosis of 1st melanoma was 58.5 years (range: 23–83). Only one patient (1.4%) was identified to have a pathogenic or likely pathogenic variant (*CDKN2A*). 14 (19.4%) patients had variants classified as variants of uncertain significance or risk alleles in other genes. Analysis of gnomAD data showed a lower frequency of pathogenic *CDKN2A* variants in the Finnish population (0%) compared to the broader non-Finnish European population (0.0545%).

Conclusion: These results suggest that clinically significant melanoma risk variants are rarer in the Finnish population compared to other European populations. The small sample size limits broader conclusions. Nonetheless, these findings support revisiting referral and testing criteria to consider inclusion of age at diagnosis.

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P01.141.A Identifying the gaps in care for neurofibromatosis Type 1 patients in Singapore

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Background: Neurofibromatosis Type-1 (NF1) is an autosomal dominant inherited condition, affecting 1-in-3000 individuals globally, caused by pathogenic variants in the *NF1* gene. Patients face higher risks of malignancies like peripheral nerve sheath tumours, breast cancer, and non-malignant features like neurofibromas and café-au-lait spots. Psychosocial impact includes body image issues, low self-confidence, and pain and predominantly studied in Caucasian populations. Addressing NF1 care in a diverse cultural and economic landscape like Singapore is crucial to bridging care gaps.

Materials and methods: We conducted semi-structured interviews with clinically or genetically diagnosed NF1 patients and analysed data using thematic analysis with elements from grounded theory.

Results: 20 patients were interviewed, the mean age being 35.0 years and 40% females. Five themes emerged regarding patient experiences: 1) NF1 trajectory begins from childhood; 2) Coming to terms with body and self; 3) Expected acceptance drives disclosure patterns; 4) Need for specialized NF1 care; 5) Building local awareness and connections. Six areas of gaps identified: 1)

Optimized multidisciplinary NF1 care; 2) Management of neurological symptoms; 3) Management of cutaneous lesions; 4) Financial coverage for NF1; 5) Early NF1 screening; 6) Local awareness and support groups. Our study aligns with similar Asian findings but highlights unique cultural factors, including Traditional Chinese Medicine use, superstitions like past-life karma, and culture-specific influences on behaviour, emphasizing their importance in NF1 care.

Conclusion: There are significant gaps in care for patients with NF1 and understanding these can lead to actionable steps for improving multi-disciplinary care and enhance quality of life.

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P01.142.B Application of MLPA method to FFPE samples with in-house developed probes

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Background: Alterations that affect wide genomic region often present in tumor cells but are difficult to detect by existing methods. Previous studies have shown the potential possibility of using MLPA for FFPE samples, however method has not been widely adopted. We tested the potency of using MLPA for FFPE in comparison with NGS/IHC.

Methods: 55 FFPE samples were collected for *ERBB2* amplification and *MET* exon 14 skipping detection. We used ExtractDNA FFPE kit and EK1-FAM SALSA Reagent Kit. 7 Self-developed probes consisting of DNA binding site, nonsense sequence, FAM-labeled primers site were used for hybridization. Capillary electrophoresis was perform via SeqStudio Genetic Analyzer, analysed with Coffalayzer.net software.

Results: All probes were successfully hybridized, ligated, and amplificated with FFPE DNA. All *HER2* amplification cases were matched with alternative methods results. We were faced with the problem of detecting *MET* exon 14 skipping and suggested possible causes and solutions.

Conclusion: FFPE material is fundamentally suitable for MLPA analysis and able to reliably detect *HER2* amplification. It is necessary to establish a cancer cellularity threshold for the possibility of using MLPA and introduce a correction factor. Our NGS data for *MET* ex14 skipping was based on cDNA analysis and for MLPA we used DNA, what could potentially lead to discrepancies between two methods. There is a problem of an unknown percentage of mutant cells among tumor cells. We plan to develop probes suitable for cDNA or selection into the site of alterations to make hybridization possible only in mutant cells in future studies.

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P01.143.C Our first experience with the commercial MSK-Access panel for liquid biopsy

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Background: Circulating cell-free DNA of an oncology patient can be used to detect somatic tumor changes. The use of ctDNA as an analyte has its limitations, as the vast majority originate from hematopoietic cells, which exploits somatic mutations, leading to its detection in cfDNA at low concentration. The MSK-Access® panel is the first commercially available liquid biopsy (LB) panel, that uses parallel sequencing of cfDNA and reference material (white blood cells) from the same collection. This approach allows the detection of somatic non-tumor changes. Precise ctDNA testing overcomes limitations of profiling in cancer tissue and have a direct impact on patient care: allows monitoring of cancer response to therapy, reveals mechanisms of drug resistance, minimal residual disease and relapse in patients.

Material and methods: MSK-Access® test allows detection of all classes of somatic genetic alterations including SNVs, indels, CNVs and structural variants in cfDNA. The test is now powered by Sophia Genetics™, uses capture and deep sequencing in 147 key cancer-associated genes. In addition to control samples, we included to validation runs NSCLC patients with point mutations, fusions and CNVs and healthy controls.

Results and Conclusion: Previously detected mutations in these patients were mostly confirmed, but some variants (AF up to 7%) were evaluated as hematopoietic cell-derived mutations and therefore false positive. Parallel sequencing of WBC with cfDNA allows better insight into the current tumor situation in oncology patients and represents precise monitoring tool during their treatment.

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P01.146.B *FH* germline testing following pathological evaluation of uterine leiomyomas with morphological features suggestive of FH deficiency: experience from a single centre

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Geneva University Hospitals, Geneva, Switzerland.

Background: Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) syndrome is a rare entity caused by germline pathogenic variants in *FH* gene. HLRCC syndrome is characterized by multiple cutaneous and uterine leiomyomas and renal cancer. In some families, pheochromocytoma and paraganglioma have also been reported.

Pathologic evaluation of uterine leiomyoma can provide an opportunity for early detection of HLRCC syndrome. Patients with suspicious pathological features results need to be referred for oncogenetic evaluation and germline testing.

Material and methods: Between 09.2020 and 12.2024, all patients with uterine leiomyomas showing distinct morphologic features for FH-deficiency underwent immunohistochemical (IHC) analysis for FH and 2SC (2-succinocysteine) proteins. Patients with loss of FH expression and/or positive 2SC staining were referred for genetic counseling and germline analysis.

Results: A total of 110 patients showed uterine leiomyomas with histologic criteria for FH-deficient morphology and 22 (20%) exhibit loss of FH expression on IHC. Among these patients, 18 had genetic counselling: 16 patients agreed to perform germline testing, 2 refused to proceed. Genetic analysis of *FH* gene identified 3 pathogenic variants and one variant of unknown significance.

Conclusion: We observed a high rate of concordance between IHC and germline testing results among patients with uterine leiomyomas showing distinct morphologic features for FH-deficiency. Not all patients harbouring germline *FH* mutations

fulfilled criteria to recommend genetic testing. In these cases, a systematic morphologic assessment for features of FH deficiency had an essential role in the identification of HLRCC patients.

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P01.147.C The coexistence of *APC* and *FH* germline variants: a case report of cooccurrence of two hereditary cancer predisposition syndromes in a single patient

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Background: It is estimated that around 10% of cancers occur through the inherited mutation in one of known cancer predisposition genes. Advances in NGS technologies have enabled a simultaneous analysis of multiple genes, resulting in increasing number of individuals diagnosed with two or more inherited genetic conditions. Among them, there are several reports of two different cancer predisposition syndromes (CPS) identified in single individuals.

Material and methods: We present the case of 19 y.o. woman with carcinoma of colon detected together with >100 polyps of large intestine. Family history was positive: her mother has had breast carcinoma and multiple polypectomy; maternal grandfather died due to colorectal carcinoma; her uncle has had colectomy due to colorectal polyposis. Based on clinical data, NGS-based Cancer panel (67 genes associated with CPS) was performed from peripheral blood in proband.

Results: The genetic analysis showed heterozygous pathogenic variants in two genes associated with CPS: c.3927_3932del (p.Glu1309Aspfs*4) in APC gene and deletion of exons 1–10 in FH gene. Target variant testing for other family members is currently ongoing.

Conclusion: Our results showed the co-occurrence of two CPS in the single individual: Familial adenomatous polyposis (FAP) and Hereditary leiomyomatosis and renal cell cancer (HLRCC). Although personal and family history was not suggestive for HLRCC, these data suggest possible benefit of multigene testing approach over target single genes analysis. All this could have important implications for improving genetic counseling, initiation of proper surveillance programs and early risk-reducing measures for all family members at risk.

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P01.149.A Syndromes of predisposition to ovarian cancer in Brazilian women: insights from a case series study

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Ovarian cancer is a major cause of mortality in gynecological cancers, largely due to late-stage diagnoses that result in low survival rates. While most cases are sporadic, 15–20% are associated with hereditary genes such as BRCA1 and BRCA2, which significantly increase the risk of cancer. Other DNA repair genes also contribute to ovarian cancer predisposition. The advent of next-generation sequencing (NGS) has facilitated broader genetic testing, enabling the identification of high-risk individuals and supporting personalized treatment approaches. This study conducted at Hospital Sírio-Libanês aims to describe genetic variants in ovarian cancer patients, emphasizing the importance of expanded genetic panels for effective disease management.

Material and methods: Data from the Hereditary Cancer Registry at Hospital Sírio-Libanês (2012–2024) was analyzed, focusing on multigene panel testing results. Among 86 ovarian cancer patients, 43 with pathogenic or likely pathogenic variants were selected. Most were from São Paulo (60.5%). Genetic testing included broad NGS panels and targeted panels for breast and ovarian cancer predisposition. Family histories were assessed using ProgenyÒ software, and data was securely stored on the REDCap platform, with ethical approval obtained.

Results: The cohort displayed various histological subtypes, predominantly high-grade serous carcinoma (79.0%). Most were diagnosed at advanced FIGO stages III and IV. P/LP variants were mainly found in *BRCA1* (40%) and *BRCA2* (33%), with others in *RAD51C, MUTYH, CHEK2*, and *TP53*. A significant 90% had a positive family history of cancer.

Conclusion: Comprehensive genetic screening using multigene panels is vital for identifying germline P/LP variants, crucial for managing ovarian cancer effectively.

C.O.I. Disclosure:

N.G. Pereira: None.

P01.150.B Correction of the major epidemiogenetic risk factors lack of physical activity and daily fiber intake in a clinical genetic setting

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Introduction: Lack of physical activity (PA < 150 minutes/week) and of dietary fiber intake (DFI < 25 g/day) are major modifiable risk factors. Medical genetics offer the opportunity to correct those factors in outpatients' clinics. Our aim was to evaluate the impact of personalized advice following standardized evaluation of PA and DFI in such a setting.

Patients & methods: Genetics adult outpatients were systematically evaluated using the International Physical Activity Questionnaire-short form and an in-house questionnaire for DFI (duration: 5 minutes), starting in 2019 and 2021, respectively, up to 2024. « Severity » was defined as <75 minutes per week for PA and <15g/day for DFI. "Improvement" was defined as progression i.e. (severity to intermediate or correction). Personalized recommendation was given to individuals below the WHO (World Health Organization) target, who were to be re-evaluated at the following consultation.

Results: Evaluation of PA and DFI revealed 39,2% (n = 714) and 71.5% (n = 263) of individuals below the WHO target, respectively. Out of those, 90.7% and 86.6% were re-evaluated after a median of 268 days (range 92–1036 days): 46.1% and 33.8%, then met the WHO target, respectively. For the most « severe » subgroup, we observed improvement for 64.7% and 50.0% respectively. Subgroup analysis for onco-genetics, CCR risk, Lynch syndrome revealed no statistical difference.

Conclusion: Systematic personalised prevention in clinical genetics practice has a large impact on major modifiable risk

factors such as PA and, to a lesser extent, DFI, with asimilar magnitude in individuals at high or low CCR risk.

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P01.151.C targeting cancer-cell mitochondria using tigecycline improves radiotherapy response in colorectal cancer cell line

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide and causes more than 50,000 deaths in the United States each year. Due to the limited therapeutic options and poor prognosis in CRC, extensive research and development of novel therapeutic methods is essential. In this regard, the presence of cancer stem cells with unlimited division ability is the main reason for the therapeutic resistance in CRC. Tigecycline is a pharmacological mitochondria inhibitor and blocks mitochondria-related cell proliferation in cancer cells. This study investigated the effects of Tigecycline combined with radiotherapy on CRC cell apoptosis.

Material and methods: Human colorectal cancer cells (HCT-116) were treated with Tigecycline and cell viability was measured with MTT assay. In the next step, the cells were exposed to radiation using a Siemens Primus 6 MV linear accelerator at radiation doses of AA, 400 cGy, BB, and DD. Finally, we evaluated cancer cell apoptosis and apoptotic-related gene expression with Annexin V/PI and gene expression.

Results: The results showed that the IC50 value of Tigecycline is 93 µM in 48 hours. Apoptosis rate and pro-apoptotic expression genes enhanced after combination therapy. Taken together, these data indicate that Tigecycline enhanced the sensitivity of cancer cells to radiotherapy and induced cell apoptosis in CRC.

Conclusion: In summary, these findings could provide new insights into cancer therapy and might be used as a novel method to improve the current state of CRC therapy.

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S. hassanpour Khodaei: None.

P01.152.D Expression of genes, connected to immunity and angiogenesis, in colorectal tumors and metastases, by Nanostring technology

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Background: The immune system plays a critical role in identifying and eliminating tumors. Dysregulation of the immune system is tightly connected to the development and spread of the cancer, together with the abnormal control of angiogenesis. During the metastatic process, cancer cells interact with the immune system and they influence each other, both in the tumor microenvironment and systemically. The objective of our study was to analyze the change in expression levels of genes, connected to immunity and angiogenesis, during the process of metastasis.

Material and methods: We used Nanostring technology for mRNA levels to compare the expression of 20 immunologic/angiogenesis genes in samples from colorectal cancer and their metastases.

Results: We detected >2 times increase of expression level in metastases compared to primary tumors for the following genes: *HIF1A*, encoding the alpha subunit of transcription factor hypoxia-inducible factor-1, the master transcriptional regulator of cellular and developmental response to hypoxia (4.8 times); *THBS1*, a potent inhibitor of T cell and dendritic cell activation (4.7); *KLF10*, involved in cell cycle regulation (2.7); *STAT6*, a signal transducer and activator of transcription (2.4); *AMD1*, encoding an important intermediate enzyme in polyamine biosynthesis (2.2); and *ITM2B*, an integral membrane protein (2.1).

Conclusion: NanoString technology allows complex profiling of mRNA expression in cancers and metastases to identify molecular mechanisms that govern metastasizing. By comparing mRNA expression in different stages of cancer, we can identify key molecules that can be used as prognostic markers for tumor vascularization and metastasis.

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P01.153.A The Prognostic Value of Liquid Biopsies for Benefit of Metastasis-targeted Radiotherapy in Relapsed Oligometastatic Prostate Cancer

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Background: Local PET/CT-guided ablative radiotherapy (RT) has been reported to improve progression-free and ADT-free survival in about 30% of patients with oligometastatic prostate cancer (OMPC) recurrence. However, no clear clinical or imaging parameters have been identified to date that allow for reliable prediction of the RT benefit. This study therefore aimed to evaluate the prognostic significance of blood-derived total RNA and circulating tumor cell (CTC)-associated mRNA for PSMA PET-guided salvage RT.

Material and Methods: A total of 44 patients were enrolled in the study between 2016 and 2020. Each patient was diagnosed with relapsed OMPC, as confirmed through PSMA- PET/CT imaging, and received RT at Radiation Oncology at Hannover Medical School. CTCs were positively enriched and RNA was extracted from liquid biopsies for real-time PCR analysis, along with miRNA sequencing for biomarker discovery.

Results: A total of 54 gene and miRNAs transcripts known to be linked to prostate cancer and treatment outcomes were selected for analysis. Significant correlations were identified between levels of these transcripts and various clinical factors, including the Gleason score, PSA levels, and UICC stage. Notably, transcript profiles of *MTCO2*, *FOXM1*, *SREBF1*, *HOXB7*, *FDXR*, and *MTRNR*, along with specific miRNAs, were associated with both temporary and long-term benefits from RT.

Conclusion: Our initial findings indicate that liquid biopsies have the potential to predict the benefits of PSMA-PET-guided salvage radiotherapy in relapsed OMPC. Further research with larger patient cohorts is required to validate these results and to establish liquid biopsy tests as a predictive tool for salvage RT.

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P01.154.B Identification of the most prevalent mutations associated with metastatic non-small cell lung cancer in the Ukrainian population

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Background: Metastatic non-small-cell lung cancer (mNSCLC) has the highest mortality rates among cancer patients worldwide (approximately 18% of all cancer-related deaths as of 2022). In Ukraine these rates are 21.1% among men and 3.4% among women. Given the lack of data on the prevalence of driver genetic alterations in the Ukrainian population of mNSCLC patients, the aim was to investigate the frequencies of these clinically significant genetic changes.

Materials and methods: All studies were conducted using next-generation sequencing (NGS) on the Ion Torrent platform (Thermo Fisher Scientific, USA). Library preparation was performed using the CleanPlex OncoZoom Cancer Hotspot Kit and the AccuFusion RNA Lung Cancer Kit (Paragon Genomics, USA).

Results: Testing was performed on biopsy FFPE samples of 79 patients with mNSCLC. Actionable findings were identified in 37 samples, representing 46.8% of the total. Among these, the EML4 (6)-ALK (20) fusion was the most common, as some patients were ALK-positive according to IHC, and NGS was used to confirm the presence of ALK gene fusions in these samples. Other most frequently mutated genes were EGFR and KRAS (G12C variant), their proportion consistent with the global statistics. Mutations in HER2 (ERBB2) and BRAF genes, as well as fusions involving ROS1 and RET genes, were also identified.

Conclusions: The results are consistent with preliminary expectations. 46.8% of patients with mNSCLC have actionable findings, some of which can only be detected by NGS. This highlights the importance of expanding the use of this method in Ukraine.

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P01.155.C A clinical audit to assess how a cancer genetic service further investigates the origin of a pathogenic/likely pathogenic *TP53* variant identified in multigene panel testing

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Background: Germline *TP53* pathogenic/likely pathogenic (P/LP) variants are diagnostic of Li Fraumeni syndrome (LFS). LFS has high cancer risk requiring rigorous screening. Clonal hematopoiesis (CH) can result in *TP53* P/LP variants that are not germline in origin. It is clinically important to determine variant etiology.

Methods: A clinical database review identified individuals with diagnostic multigene panel testing (January 2021 - June 2024) and a P/LP *TP53* variant identified. Records were reviewed to assess underlying etiology.

Results: Eleven individuals with P/LP *TP53* variants were identified. See Table 1 for demographics and variant allele frequency (VAF). Table 2 summarizes outcome of investigations to determine etiology of *TP53* P/LP variant. All female patients with LFS had breast cancer. Only one patient (number 2) met Chompret criteria, while one patient with mosaic LFS (number 9) met modified Chompret criteria.

Conclusion: The etiology of a P/LP TP53 variant was resolved in all cases. Variants with VAF >40% were not further investigated, in keeping with recommendations. In our population 63,6% were heterozygous P/LP TP53, 18,2% mosaic and 18,2% CH. Individuals with germline P/LP TP53 variants often did not meet clinical LFS testing criteria.

Table 1:

Patient number	Sex assigned at birth	Cancer diagnoses at time of testing	Age of onset	OriginalSample	VAF (%)
1	Female	Papillary Thyroid	47	Saliva	46.7
		Breast	48		
2	Female	Breast	40	Saliva	53.4
3	Female	Thymoma	27	Blood	48.6
		Breast	39		
4	Male	Osteosarcoma	11	Saliva	47.5
5	Female	Bilateral breast	47	Saliva	49.0
6	Male	Colon	50	Saliva	52.0
		Leiomyosarcoma	54		
7	Female	Breast	42	Saliva	41.0
8	Female	Breast	42	Saliva	17.0
9	Female	Breast	27	Saliva	18.0
10	Female	Bilateral breast	48	Saliva	12.0
11	Female	Breast	43	Saliva	34.1

Table 2:

Patient number	Additional sample tested	Type of sample	TP53 variant detected	VAF (%)	Final diagnosis given
1	No	-	-	-	Germline LFS
2	No	-	-	-	Germline LFS
3	No	-	-	-	Germline LFS
4	No	-	-	-	Germline LFS
5	No	-	-	-	Germline LFS
6	No	-	-	-	Germline LFS
7	No	-	-	-	Germline LFS
8	Yes	Skin	Yes	10.0	Mosaic LFS
9	Yes	Hair	Yes	9.0	Mosaic LFS
10	Yes	Hair	No	-	CHIP *
11	Yes	Hair	No	-	CHIP *

*Cannot rule out somatic mosaicism.

C.O.I. Disclosure:

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P01.157.A The contribution of microarray analysis to diagnosis, risk classification, and treatment in leukemia patients

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Background: Accurate genomic characterization is essential for leukemia classification, risk stratification, and treatment planning. However, karyotyping and FISH have limitations due to their low resolution or target specificity. We utilized SNP-array to enhance genomic characterization by identifying copy number variation (CNV) and copy-neutral loss of heterozygosity (CN-LOH). Our aim is to assess the feasibility of integrating SNP-array into routine clinical practice.

Material and methods: Archived DNA samples from 46 leukemia patients (20 ALL, 17 AML, 3 CLL, 6 other leukemia types) with inconclusive routine genetic test results were analyzed via SNP-array to identify CNV and CN-LOH.

Results: Clinically significant (tier 1 and tier 2) alterations were identified in 78.2% of cases. Diagnostic genetic markers were detected in 4 cases (8.7%), prognostic in 24 cases (52.2%), and therapeutic in 1 case (2.2%). In B-ALL cases, *IKZF1-plus* alterations (*IKZF1*, *CDKN2A*, *PAX5*), atypical iAMP21, *P2RY8::CRLF2* fusion, and *RB1* deletion were found. T-ALL cases showed *STIL::TAL1* fusion, *CDKN2A* deletion with 9p CN-LOH, and *PTEN* deletion with 10q CN-LOH. AML cases revealed *ERG* amplification, *NF1* deletion, 13q CN-LOH, 17p deletion, and chromoanagenesis-like complex changes. CLL cases showed varying sizes of 13q deletions and 2p gain. A JMML patient with a germline *CBL* mutation had two CN-LOHs with different clonality detected at 11q.

Conclusion: SNP-array enhances genetic characterization, particularly in ALL cases and AML cases with inconclusive genetic test results. However, clonal variations, low clonality amplifications, and balanced rearrangements remain undetectable. Therefore, SNP-array should be used alongside karyotyping and FISH for a comprehensive genomic profiling.

C.O.I. Disclosure:

M. Acar: None. **S. Altiner:** None. **H. Ilgin Ruhi:** None.

P01.158.B Cancer molecular profiling in Northern Bergamo patients: focus on the double alterations and microsatellite instability

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Background: Currently, the advent of precision oncology is rapidly revolutionizing both the diagnosis that the treatment of cancer through more oriented therapy. Here, we investigated the cancer molecular profiling in Northern Bergamo patients.

Material and methods: A cohort of 104 cancer patients have been the subject of this investigation.

Results: On the total individuals the 76.9% was mutated while the remaining 23.1% no mutation was identified. The number of mutations found were 82, of which the 96.3% were missense variants. The major variants identified were present in KRAS (25%), PIK3CA (11.5%) and BRAF (8.7%) genes. C.35G>T (42%) exon 2 in KRAS, c.1799T>A (44%) exon 15 in BRAF and c.263G>A (42%) exon 2 in PIK3CA genes were the three major alterations expressed. Geografic distribution investigation demonstarted that in the Upper-Valley the percentage of the mutated and the wild type samples identified were respectively of 68% and 32%. The most common type of tumor was represented by colorectal adenocarcinoma (43%) and the prevalent missense variant was represented by KRAS exon 2 c.35G>T mutation (56%). Instead, in the Lower-Valley was the melanoma (32%), with associated BRAF c.1799T>A gene mutation (50%). Finally, 63-year-old female cancer patient was selected as clinical case, reporting a double genetic alteration with associated MSI instability.

Conclusion: The knowledge of mutational spectra is very important to evaluate the most recurrent variants. The implementation of the genetic screening of different cancer populations in several areas can certainly lead benefits to the patient as an earlier diagnosis and reduction of treatment times.

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P01.160.D Molecular profiling of non-small cell lung cancer in the brazilian population

Bruno da Silveira Corrêa.[1], *Anelise Baptista da Silva*[1], ***Claudia Paiva Nunes***[1], *Julia Crispim da Fontoura*[1], *Mariana Mazza Santos*[2], *Thais Assis*[2], *Maristela Ocampos, PhD*[2], *Cristina Beatriz Cazabuena Bonorino*[1]

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Background: Lung cancer is the leading cause of cancer-related mortality worldwide, with 1.8 million deaths in 2022. Molecular biomarkers are crucial for clinical management in oncology. However, the mutational landscape of non-small cell lung cancer (NSCLC) in Brazil is poorly characterized. This study aims to profile NSCLC mutations in Brazil, correlate them with clinical data, and explore underlying mechanisms.

Material and methods: Samples from 182 NSCLC patients were collected at the Santa Casa de Misericórdia Hospital Complex in Porto Alegre, Brazil, and preserved in paraffin. DNA extraction was performed with Quick-DNA FFPE Miniprep kit (Zymo Research), library preparation with Library Preparation EF 2.0 kits (Twist Bioscience). Sequencing on the MiSeq platform (Illumina) used a custom panel targeting 29 clinically actionable genes. Additionally, sequencing is under way at MercoLab Diagnósticos using the Oncomine Comprehensive Assay Plus DNA panel (Thermo Fisher Scientific), with over 500 cancer-related genes. Data analysis is being carried by custom scripts to identify genetic alterations and correlate them with clinical data.

Results: Broad panel sequencing of seven patients identified mutations in cancer driver genes EGFR (2/7), KRAS (1/7), PIK3CA (1/7), and TP53 (2/7), along with pathogenic variants in ERBB2, RB1, PTEN, HRAS, RAD51B, RAD54L, PIK3CA, TERT, and MET. None had microsatellite instability, and one had an intermediate tumor mutational burden of 11.42 mut/Mb.

Conclusion: This study is among the first to comprehensively analyze the mutational profile of NSCLC in Brazil, including both common lung cancer-associated genes and other relevant molecular alterations.

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P01.161.A Molecular genetics vs molecular cytogenetics in a B ALL diagnosis

Mirjam Lista[1], *Serena Albertazzi*[1], *Federica Estersini*[1], *Kokou E.A. Ayissou*[1], *Chiara Milanesi*[1], *Nicla Manes*[1], *Alessia Campagna*[2], *Rossella Caselli*[1]

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Acute B lymphoblastic leukemia is a hematological malignancy characterized by lymphoblasts uncontrolled proliferation. Advances in genomic profiling techniques have identified more specific genetic alterations and mutations driving B-ALL, leading to the development of novel therapies. In adults, common genetic alterations investigated include *KMT2A* rearrangements, *ETV6::-RUNX1, TCF3::PBX1, TCF3::HLF, BCR::ABL1* translocations [1,2].

We present the first adult case in our cohort, where a canonical *BCR::ABL1* rearrangement is identified only with genetic molecular analysis and not with FISH. FISH analysis was performed using a specific probe on uncultured material following direct nuclear extraction to search for gene targets.

FISH revealed only the presence of *HLF*, *BCR*, *PBX1*, and *KMT2A loci* in triple and quadruple copies, respectively excluding any rearrangements. These results indicated a signal pattern compatible with a ploidy alteration also investigated using oligo-SNPs array. However, for the purpose of a specific genetic characterization, the obtained information were not conclusive. Further molecular investigations, conducted via RT-PCR, were carried out to detect the BCR::ABL1 alteration, revealing the p190 canonical transcript. In contrast to all the cases we have seen, where FISH analysis yields the expected results this finding led us to hypothesize a cryptic rearrangement with probable chromothripsis.

We have not yet been able to identify the genetic-molecular cause that masked the presence of the gene fusion in the cytogenetic analysis: this is our future prospective. This case pinpoints the importance of combining cytogenetic and molecular genetic analyses in all B-ALL cases to identify all rearrangements and to ensure no alterations are missed.

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P01.162.B The *BIRC5* connection: gene polymorphisms and survivin isoforms in oral and oropharyngeal squamous cell carcinoma

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Background: Oral and oropharyngeal squamous cell carcinoma are the most common head and neck cancers. The main risk factors are alcohol and tobacco use, as well as HPV infection. Most patients with early-stage cancer can be cured, but patients with advanced disease, which make two-thirds of new diagnoses still have the five-year survival rate of about 60%. Survivin, encoded by *BIRC5* gene, has been implicated in head and neck cancer development. The aim of this study was to investigate *BIRC5* polymorphisms and expression of different isoforms in head and neck cancer.

Material and methods: 48 patients (31 with oral and 17 with oropharyngeal squamous cell carcinoma) and 45 controls (patients who were treated for non-malignant diseases) were included in this study. Genomic DNA was isolated from blood, and RNA was isolated from tumors or healthy mucosa. The entire coding region of *BIRC5* gene, as well as promoter and 3'UTR regions were genotyped for polymorphisms using high resolution melting analysis and Sanger sequencing. The expression of eight different survivin isoforms were determined by qPCR.

Results: Seventeen *BIRC5* polymorphisms were found. Three were found only in patients and two only in controls. Eleven polymorphisms showed minor allele frequency (MAF) greater than 5%. All examined isoforms were detected in tumor samples, while two isoforms were not detected in healthy mucosa. All isoforms were statistically significantly more expressed in tumor tissue, with the most expressed isoforms being wild type, 2α and 2B.

Conclusion: *BIRC5* polymorphisms were associated with expression of only few isoforms.

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I. Mumlek: None. **M. Sabol:** None. **P. Ozretić:** None. **L. Glavaš-Obrovac:** None. **V. Musani:** None.

P01.163.C Next-generation sequencing reveals multiple *ATM* gene variants in urothelial bladder cancer

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Background: Urothelial bladder cancer (UBC) is the most common urinary malignancy and the tenth most diagnosed cancer worldwide. Depending on the muscle infiltration, UBC can be classified as muscle-invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC). Based on its heterogeneity, comprehensive genomic studies reveal significant molecular differences.

Material and methods: The study included 32 patients with bladder cancer, 13 with MIBC and 19 with NMIBC. Genomic DNA was extracted from fresh frozen tumor samples and sequenced by NextSeq (Illumina) using customized panel of 95 genes. Only the pathogenic, likely pathogenic and variants of unknown significance were evaluated.

Results: The most frequently affected genes, present in ≥12% of the samples are: *TP53* (44%), *FGFR3* (41%), *PIK3CA* (34%), *STAG2* (19%), *ATM* (16%), *ERBB2* (16%), *APC* (12%), *BRAF* (12%), *KRAS* (12%) and *TERT* promoter (12%). The result is in line with the previously proposed molecular stratification by mutations in *TP53/MDM2* pathway, *FGFR3*, and *RAS* genes. Interestingly, we observed *ATM* variants in five samples (26%) of the NIMBC, but none in the MIBC subgroup. In two samples we detected single variants (missense and frameshift deletion), while three samples had multiple variants in this gene. *ATM* plays a central role in cell division and DNA repair.

Conclusion: Precise pathological classification together with clarification of the molecular subtype, can significantly contribute to understanding the entity of a given tumor and, especially in early stage lead to improved individualized treatment in terms of the existing and novel therapies that would target certain molecular pathways.

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P01.164.D A case report of two compound heterozygous *CHEK2* variants presenting with ovarian and breast cancer

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Background: Breast cancer remains the most prevalent cancer among women worldwide, with early diagnosis significantly improving prognosis. Genetic predisposition plays a crucial role in breast cancer risk. Mutations in *CHEK2* have been linked to a moderately increased risk of several types of cancer, particularly breast, prostate, and colorectal cancers, highlighting its role as a tumor suppressor. While monoallelic mutations in *CHEK2* and their relation to hereditary cancer syndromes are well-published, much less is known about biallelic mutations in *CHEK2* and their associated cancer risks remain under-explored.

Materials and methods: We present the case of a 66-year-old woman diagnosed with breast cancer (ER: 90%; PR: 0%) aged 62 and bilateral serous ovarian cancer at the age of 65 years. She received genetic counselling at the certified Center for Familial Breast and Ovarian Cancer at the University Medical Center Schleswig-Holstein Campus Luebeck and underwent genetic testing, revealing the following compound heterozygous variants in the *CHEK2* gene: c.(908+1_909-1)_(1095+1_1096-1)del (p.?) and c.1100delC; p.(Thr367Metfs*15). These variants were identified using next-generation sequencing, integrated CNV analysis, and Multiplex Ligation-dependent Probe Amplification. Moreover, long-range PCR and sanger-sequencing showed a *trans* configuration of the variants.

Results: The genetic profile of the patient suggests a possible increased cancer risk associated with biallelic *CHEK2* mutations, particularly in relation to breast and ovarian cancers.

Conclusion: The case highlights the importance of investigating biallelic *CHEK2* mutations in patients with multiple cancers. Further research is necessary to understand the implications of these mutations on cancer risk and clinical management.

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P01.165.A *PDCDL1* gene variants are associated with invasive features of Malignant Melanoma

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Background: The incidence of malignant melanoma (MM) continues to increase annually, and its prognosis rapidly worsens as the disease progresses. Single nucleotide polymorphisms (SNPs) have become key tools in the study of cancer genetics, influencing susceptibility and prognosis.

Material and methods: We analyzed the relationship between SNPs of the *PDCDL1* gene and the prognosis and tumor invasiveness of MM, considering clinicopathological characteristics in a total of 377 whole blood samples from MM cases.

Results: Patients with genotypes CG or GG at rs822336 (OR = 3.01, 95%CI = 1.53–5.92; p = 0.0017), TA or TT at rs822337 (OR = 2.45, 95%CI = 1.22–4.93; p = 0.0098) and CT or CC at rs822338 (OR = 2.23, 95%CI = 1.05–4.73; p = 0.028) had a higher risk of developing invasive melanomas compared to those with in situ melanomas. Patients with genotype AG or GG at rs2297136 had a lower risk (OR = 0.29, 95%CI = 0.11–0.75; p = 0.0038) of invasive MM. Genetic analysis at the haplotype level yielded similar results (OR = 2.95, 95%CI = 1.08–8.10; p = 0.036) for the GTCAC haplotype (rs822336 rs822337, rs822338, rs2297136, rs4143815). In addition, patients carrying the AA homozygous genotype at rs2297136 had thicker tumors than AG or GG carriers (p = 0.030). No significant association was found between the studied SNPs and melanoma-specific survival (MSS) or progression-free survival (PFS) in patients with disease progression.

Conclusion: This study suggests that SNPs rs822336, rs822337, rs822338 and rs2297136 of the PDCDL1 gene are associated with an increased risk of invasiveness and tumor thickness in MM cases. Further studies on SNPs considering genetic and epigenetic factors are needed to better understand MM susceptibility and its impact on prognosis.

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P01.166.B Heritable TP53-related cancer syndrome (hTP53rc syndrome)/Li-Fraumeni syndrome (LFS) in Poland. Genotype-phenotype analysis

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Background: *Li-Fraumeni syndrome (LFS; OMIM #151623) is caused by pathogenic variants in the TP53 gene*. Typical spectrum of LFS: soft-tissue sarcomas, osteosarcomas, adrenocortical carcinomas, CNS tumours and breast cancers before 31 years. If Chompret

modified criteria are met and/or a patient has been diagnosed with hypodiploid acute lymphoblastic leukaemia or otherwise unexplained sonic hedgehog-driven medulloblastoma or jaw osteosarcoma, testing for germline TP53 pathogenic variant should be offered.

Material and methods: 20 patients with germline pathogenic/likely pathogenic variants in TP53 gene have been identified. The patients received detailed genetic counselling and were offered surveillance programme, according to the guidelines. DNA had been extracted from the peripheral blood leukocytes. Causative genetics variants revealed by NGS were confirmed by Sanger sequencing in independent analysis. The pathogenicity of the variants had been assessed according to the Recommendations from the ClinGen TP53 Expert Panel.

Results: Pathogenic/likely pathogenic variants: c.455C>T, c.473G>A, c.497C>G, c.541C>T, c.631_632dup, c.637C>T, c.721T>G, c.733G>A, c.742C>G, c.743G>A, c.772G>A, c.808T>C, c.818G>A, c.845G>A. Tumor spectrum: breast cancer (12 patients, 5 cases were >31 years), soft tissue sarcoma (leiomyosarcoma, undifferentiated pleomorphic sarcoma), osteosarcoma (1), osteochondroma (1), choroid plexus carcinoma (1), melanoma (2), papillary thyroid cancer (1), serous ovarian cancer (1), lung cancer (1 adenocarcinoma).

Conclusion: We found 14 different mutations in 20 patients coming from 17 unrelated families, We were not able to prove any founder effect in this group. Our study suggests that LFS can not be excluded in breast cancer patients older than 31 years, Further studies in larger groups are needed.

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P02 Reproductive Genetics

P02.001.C PGT results in double biopsied embryos - rate of inconsistency

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Background: Chromosomal aberrations, such as whole-chromosome aneuploidies, segmental aneuploidies, whole-chromosome mosaicism, and segmental mosaicism, are key factors influencing embryonic development and the outcomes of fertility programs. Despite the higher probability that aneuploid embryos will arrest at the cleavage stage, embryos with chromosomal abnormalities can still form blastocysts and are often morphologically indistinguishable from chromosomally normal (euploid) embryos, especially when evaluated by static observations. The aim of our study was to analyse the chromosomes from two separate biopsies in the same embryos in order to determine the rate of concordance in chromosomal set.

Material and methods: Totally, 15 embryos were doubling biopsied and subjected to WGS chromosomal testing.

Results: Consistently euploid embryo was discovered in 4 out 15 samples (27%), in 5 samples (33%) one biopsy showed euploid and the second one - aneuploid (1 sample), mosaic (1 sample) or chaotic embryo (3 samples). In two embryos both biopsies showed aneuploidy, two were consistently chaotic and two with aneuploidy in the first biopsy and chaotic embryo from the second one. We are doing clinical follow-up for all analysed embryos.

Conclusion: There was high rate of chaotic embryos (30%) when double biopsy was performed - technical implementations should be considered. Despite the implementation of pre-implantation genetic screening and other advanced in vitro fertilization (IVF) techniques, the identification of aneuploid embryos remains complicated by high rates of mosaicism.

C.O.I. Disclosure:

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P02.003.A Preimplantation genetic testing without invasive embryo biopsy (Award Candidate)

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Background: Preimplantation genetic testing (PGT) is an assisted reproductive technology that requires an invasive embryo biopsy. Spent culture medium (SCM) from in vitro cultured embryos contains cell-free DNA, offering a non-invasive testing alternative. Existing non-invasive PGT (niPGT) methods focus on detection of aneuploidy and fail to address the meiotic/mitotic origin of aneuploidies and mosaicism, limiting clinical applicability. We developed an niPGT method for simultaneous detection of monogenic disorders, and aneuploidies, including their segregational origin.

Material and methods: We analyzed 90 SCM samples from 53 blastocysts (14 families) affected by monogenic disorders. Five amplification protocols were tested, followed by whole-genome sequencing. Genome-wide haplotypes were reconstructed by family-based haplotyping using haplarithmisis, optimized for cell-free DNA. Haplotypes and copy number profiles were compared to biopsy results.

Results: Genome-wide haplotypes and copy number profiles were generated and compared, allowing selection of the optimal amplification method. In 74 samples, the sex identified in medium matched biopsy, while 16 showed mismatches, suggesting (maternal) contamination. High-quality embryos assessed with the optimal amplification method had a median haplotype concordance of 92.5% ([89.83–95.27], $n = 15$). Notably, all meiotic aberrations ($n = 3$) in biopsy were also observed in medium, while mitotic aberrations were inconsistent, which is inherent to mosaicism.

Conclusion: Our niPGT method distinguishes mitotic aberrations from meiotic aberrations and tests for monogenic disorders non-invasively, offering a new paradigm for embryo selection. Future improvements in contamination control will further advance the clinical applicability of niPGT. Our approach enables

non-invasive genetic testing and could be extended to other medical disciplines, such as oncology.

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P02.004.B Hereditary cancer-linked germline variants are significantly enriched in infertile men

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Backgroud: Epidemiological studies show infertile men and their families have a higher tumor risk, suggesting shared genetic etiology. The aim of the study was to analyze hereditary cancer-linked germline variants in 522 infertile compared to 323 fertile men.

Material and methods: The ESTonian ANDrology (ESTAND) cohort was recruited and phenotyped at the Andrology Clinic of Tartu University Hospital. Male infertility was defined by total sperm count ≤39 million/ ejaculate; fertile men were normozoospermic and partners of pregnant women. Whole exome sequencing data was assessed for disease-causing variants in 157 hereditary cancer genes. The custom data analysis pipeline (1,2) considered in silico predictions of variant pathogenicity, previous literature, available database entries and clinical data.

Results: The ESTAND cohort revealed a nearly five-fold enrichment ($P = 2.3 \times 10E\text{-}4$) of hereditary cancer-linked variants in infertile versus fertile men. No significant difference in findings was observed between azoospermia and oligozoospermia cases. The majority of men with findings carried variants in DNA repair genes, most in Fanconi Anemia pathway genes such as *BRCA2, FANCM* and *BRIP1*. Six men had early adulthood cancers corresponding to their genetic finding, and most with pedigree data showed familial cancer incidence. Most affected genes are highly expressed in testicular cells, with mutant mouse models showing male infertility and cancer risk.

Conclusion: Hereditary cancer variants may disrupt spermatogenesis due to shared defective mechanisms responsible for genome integrity, explaining infertile men's higher cancer risk. Early knowledge of germline predisposition to cancer enables multidisciplinary management options, supporting routine hereditary cancer gene analysis in spermatogenesis disorders.

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P02.005.C Clinical application of whole exome sequencing in the diagnosis of men with severely impaired spermatogenesis

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Background: Since monogenic causes of males are still largely unexplored its testing is primarily applied to research settings and has not been yet incorporated into clinical practice [1]. With the rapid accumulation of new candidate genes associated with male infertility, the question remains regarding the extent to which monogenic testing could improve the clinical diagnosis of infertile men.

Material and methods: Based on systematic literature screening, we designed a panel of candidate genes associated with severe male infertility. We tested the 420-gene panel on whole exome sequencing data from a representative multicentric cohort of 240 men with significantly impaired spermatogenesis, including men with non-obstructive azoospermia, and severe oligozoospermia (<5 million spermatozoa/ml). Identified variants were classified using the ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020 [2].

Results: We identified a potential monogenic cause in 6.7 % of infertile men. With the detection of likely pathogenic/pathogenic variants in *ADAD1, GALNTL5, MSH4, RBMXL3, RHOXF2B, SPOCD1, SSX1, STAG3, STRA8, TDRD9, TEX14*, and *TEX15* we contributed further evidence regarding the role of monogenic causes in severe idiopathic male infertility.

Conclusion: The identification of a potential monogenic cause in 6.7 % of infertile men confirmed the utility of monogenic testing in men with severely impaired fertility. Incorporation of monogenic testing into the diagnostic process of infertile men would improve both the understanding and management of infertile men. Improved diagnosis would benefit the patients, by reducing the need for unnecessary invasive tests and help inform the patients about their reproduction options.

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P02.006.D LINE-1 methylation sustains telomere length : effects on pregnancy

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Background: Early pregnancy loss (EPL) is defined as a spontaneous pregnancy termination within the 12th week of gestation whose exact causes remain unexplained in approximately 50% of cases. Fertility rate decreases as maternal age increases and epigenetic age-dependent conditions may favor miscarriage. We aim to investigate DNA methylation and telomere length as predictors of EPL risk.

Material and methods: Telomere length (T/S) and LINE-1 (%), as surrogate of global DNA methylation, have been assessed in a cohort of 242 pregnant women by comparing EPL (n = 129) with voluntary interruption (VPI, n = 113).

Results: Telomere size and methylation rate decreased as the age of women increased with EPL group having significantly lower values (T/S: 322.6 ± 142.0 versus 455.0 ± 290.6; $P < 0.000001$ and LINE-1 (%): 81.66 ± 4.2 versus 86.01 ± 3.7; $P < 0.000001$) characterized by stronger age-dependent lowering compared to VPI ($P < 0.001$). In correlation analysis, EPL were overrepresented in the area below the 25th percentile of the T/S and LINE-1 distribution (89.5%), while VPI controls were dramatically overrepresented in the area above the 75th percentile (94.0%). Comparing EPL/VPI ratio in these two extreme areas (EPL/$VPI_{(<25th)} = 3.12$ versus EPL/$VPI_{(>75th)} = 0.32$), a significant risk of early pregnancy failure was obtained when women carried both low telomere size and LINE-1 methylation (OR = 9.70, 3.94–23.72; $P < 0.0001$).

Conclusion: The combined effect of short telomeres and low methylation worsens the pregnancy maintenance, proposing such epigenetic age-related biomarkers as informative for pregnancy outcome and, in turn, fertility longevity. Their employment in the clinical practise has the potential to become an applicable tool also for reproduction technologies.

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P02.007.A Exome sequencing of 1013 cases of disorders/differences of Sex Development (DSD) - results of a 16 year study

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Background: DSDs include a wide range of pathologies that impact gonad formation, development and function in both 46,XX and 46,XY individuals. Here, we summarize our experience of 16 years of exome sequencing performed on 1013 DSD cases.

Methods: During the period 2009–2024, WES was performed on 46,XY DSD (n = 764; gonadal dysgenesis, testicular regression sequence, anorchia, or unexplained undervirilized male) or 46,XX DSD (n = 249; ovarian dysgenesis, MURCS, cloacal extrophy, vaginal atresia, ovotesticular/testicular DSD).

Results: WES established a genetic etiology in 36.8% of the 46,XY cohort (30.9 %/non-syndromic cases and 60.4%/syndromic cases). The most common genes involved were - *GLI3, WT1, CHD7* (syndromic) and *AR, SRD5A2, NR5A1, HSD17B3* and *DHX37* (non-syndromic). 4% of individuals in 46,XY DSD cohort showed evidence for digenic/oligogenic inheritance. In the 46,XX DSD cohort the overall genetic diagnostic yield was 29.3% with 26.7% yield for ovotesticular/testicular DSD cases. In this group pathogenic variants were identified in sex-determining genes (e.g. *SRY, NR2F2, WT1, RSPO1, WNT4*) or genes required for ovarian maintenance (e.g. *NOBOX, MCM8, FILGA, TP63*).

Conclusions: WES has identified many new genetic causes of DSD (e.g *ZFPM2, ZNRF3* etc). However, pathogenic variants in each gene affect a relatively small number of individuals (e.g. *DMRT1, GATA4, ZNRF3*). Consequently, the number of genes causing DSD continues to increase without a proportionate augmentation of the overall diagnostic yield. Most children with DSD still do not have a genetic diagnosis. A significant proportion of these unexplained cases are likely due to variants in regulatory elements of genes required for gonad formation.

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P02.008.B Quality control in preimplantation genetic testing for aneuploidies: beyond Vienna consensus

***Andrei Kullyev[1]**, Svetlana Avdeichik[1], Andrei Kartuesov[1], Tatiana Iunik[1], Kamola Khalilova[1], Irodakhon Shoyusupova[1], Malika Temirgalieva[1], Maftuna Islambekova[2], Lasha Nadirashvili[3], Veniamin Kazarinov[3], Sergei Sergeev[3], Yaron Goikhman[1]*

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Background: Vienna consensus sets ≥90% of successfully diagnosed embryos as a competency value and ≥95% as a benchmark value in preimplantation genetic testing for aneuploidies (PGT-A). The percentage of embryos diagnosed as euploid is a critical parameter in PGT-A, as it determines the cumulative live birth rate and the efficiency of PGT-A. Yet, no benchmark has been set for this parameter. False positive results could lead to some euploid embryos being diagnosed as mosaic or aneuploid. The percentage of euploid embryos varies widely between laboratories. Published studies show that changes in the analysis of the PGT-A results could increase the percentage of embryos diagnosed as euploid, indicating that previously, some euploid embryos were diagnosed as mosaic and aneuploid.

Material and methods: 4,500 samples were analyzed with the EmbryoMap PGT-A solution on the Illumina sequencing platform. Embryo biopsy and cold chain logistics were optimized for the best sample quality. Sample processing and results analysis were optimized to avoid false positives.

Results: The percentage of successfully diagnosed embryos increased from 96.5% to 99.0%. The percentage of embryos diagnosed as euploid reached 68.2% for all age groups (up from 31.1% with our previous workflow with outsourcing to an outside

laboratory) and 73.2% for patients under 35 years old (up from 43.9%).

Conclusion: For modern PGT-A, the benchmark value for successfully diagnosed embryos could be increased from 95%. To control the efficiency of PGT-A, a benchmark for the percentage of euploid embryos in the reference group under 35 years old is needed.

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P02.009.C The transcription factor *TBX3* is mutated in congenital hypogonadotropic hypogonadism

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Background: CHH is a heterogenous genetic disorder characterized by absent puberty and infertility. Mutations in more than 50 loci have been identified to underlie the disorder, yet 60–70% cases remain unsolved. Therefore, we performed unbiased genetic studies to discover novel CHH gene and implication in puberty.

Methods: Analysis performed: (i) gene burden test (476 CHH vs 800'000 controls from gnomAD) for gene variants with MAF< 0.01% ($p = 2.5 \times 10$^-6); (ii) screening for rare variants in *TBX3* in our CHH cohort (iii) bioinformatic screening to identify miRNA regulators of *TBX3*; and (iv) ICV injection of mimetic miRNA-137 in rats (postnatal d21-34) to evaluate pubertal status.

Results: Rare variants in *TBX3* were significantly enriched in CHH vs controls. 13 rare variants in *TBX3* were identified in 13 CHH probands, located in different protein domains. Notably, we identified 6 loss-of-function (LoF) variants in *TBX3*, including a de novo (p.R131*). Additionally, *Tbx3* is expressed in migrating GnRH neurons and inhibition of *Tbx3* by miRNA-137 in prepubertal rats led to delayed puberty.

Conclusions: Through unbiased genetics, we identified *TBX3* as a putative CHH gene, and the presence of 6 LoF variants is consistent with haploinsufficiency. *TBX3* is a transcription factor critical to POMC and KISS1 neurons and is mutated in Ulnar Mammary Syndrome (UMS). Notably, CHH and UMS appear to share clinical features (*e.g.* genital abnormalities, delayed puberty, etc.) and a potential genetic overlap. The link between *TBX3*, KISS1 neurons and miRNA-137 is currently under investigation.

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P02.010.D Known and novel monogenic causes of cryptorchidism

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Background: Cryptorchidism (CR) is a congenital malformation where at least one testicle is missing from the scrotum. Occurring in 1–2% of newborn boys[1], it may lead to spermatogenic failure (SPGF; $<39 \times 10^6$ sperm/ejaculate) and/or testicular cancer later in life. CR has several risk factors, including likely pathogenic (LP) and pathogenic (P) variants in testicular descent genes (e.g. *INSL3*). This study aimed to identify disease-causing variants in gonadal development genes within the ESTonian ANDrology (ESTAND) cohort, including Ras/MAPK pathway genes as a novel aetiology for CR.

Material and methods: Whole-exome sequencing was performed on 844 ESTAND men (521 infertile, 323 fertile) recruited at the Andrology Clinic, Tartu University Hospital. Disease-causing variants were studied in 61 gonadal development candidate genes, applying a custom filtering pipeline, while also considering clinical, pedigree, and genotype-phenotype data of the subjects.

Results: LP/P variants were noted in 14/61 genes. Seven known gonadal development genes had 20 different LP/P variants among 18 infertile and two normozoospermic men. CR was present in 12 SPGF cases. The most prevalent genes were *NR5A1*, *DHX37*, *DMRT1*, *AR*[2].Nine SPGF and one subfertile individual carried 10 LP/P variants in seven Ras/MAPK genes[3]. None had been previously diagnosed with RASopathy. The prevalence in CR cases with RASopathies versus the general population was 40-fold higher.

Conclusion: This study shows a link between CR and LP/P variants in several gonadal development genes. Furthermore, Ras/MAPK pathway presents a novel aetiology of male infertility and congenital CR.

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P02.011.A Using SNP-array based loss of heterozygosity analysis to identify candidate genes in premature ovarian insufficiency

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Background: Premature ovarian insufficiency (POI), defined as a decline in ovarian function before age 40 evidenced by amenorrhea and hormonal criteria, affects 2–4% of women and leads to infertility, hormonal insufficiency, and various long-term

health risks. Although several genetic, autoimmune, and iatrogenic factors have been implicated, the genetic architecture of POI remains elusive. We aimed to examine the contribution of loss of heterozygosity (LOH) in women with POI, leveraging SNP-array data to identify susceptibility regions and candidate genes.

Material and methods: We analyzed, through Affymetrix 750k SNP-arrays, 393 women with POI and 759 controls consisting of pregnant women with comparable age, referred to our laboratory between 2017 and 2023. LOH segments were defined as segments of a unique genotype spanning 3 Mb or larger containing at least 25 consecutive markers. We applied Fisher's exact tests in a 1 Mb sliding window, restricting analyses to regions with a ratio of POI vs. healthy above 3.

Results: Women with POI displayed a higher mean total LOH length than controls (47.3 Mb vs. 33.1 Mb, $p < 0.01$), with an overrepresentation of chromosome 4 (6.6% vs. 3.8% in POI vs. control respectively, $p < 0.003$). Individuals presenting with a total LOH exceeding 65 Mb had a higher risk of POI (odds ratio = 3.37, [2.22–5.12], $p < 0.0001$). We identified 56 significantly overrepresented LOH regions and, using GTEx data and standardized bibliographic searches, we extracted 45 candidate genes.

Conclusion: We demonstrate significant LOH overrepresentation in POI. The identified regions and candidate genes provide valuable insights into POI candidate genes and genetic risk factors.

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P02.012.B Multi-omics profiling of human ovarian granulosa cells uncovers cell type-specific regulatory landscapes

Charlotte Matton[1], *Julie Van De Velde*[2], *Quinten Mahieu*[1], *Lisa Hamerlinck*[1], *Lara Colombo*[1], *Sarah Vergult*[3], *Alexandre Segers*[1], *Mattias Van Heetvelde*[1], *Seoyeon Oh*[1], *Celine Everaert*[1], *Kelly Tilleman*[2], *Dominic Stoop*[2], *Eva D'haene*[1], *Elfride De Baere*[3]

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Background: Granulosa cells (GCs) are key in the ovarian somatic compartment, supporting folliculogenesis and ovarian maintenance. They maintain an intricate relationship with oocytes, requiring a strict spatiotemporal regulation by GC-specific transcription factors, such as FOXL2. Dysregulation of these transcription factor genes is implicated in the pathogenesis of primary ovarian insufficiency (POI), affecting 1% of the female population and representing a major cause of female infertility. Here, we mapped 3D chromatin architecture and regulatory landscapes governing gene expression in adult human GCs.

Methods and results: Primary cumulus (surrounding the oocyte) and mural (outer layer of the follicle) GCs were collected from adult females (n = 17) who were subjected to assisted reproduction. Upon isolation, GC identity was confirmed via hormonal assays, GC-specific marker profiling (qPCR and immunocytochemistry) and FSHR+ cell flow cytometry. Short-read total RNA-seq and deconvolution analysis revealed diverse cellular composition of the primary GC samples, with GCs constituting up to 85%. Subsequently, we used an integrative multi-omics approach to profile transcriptional regulation in primary GCs. Epigenomic profiling through CUT&RUN (H3K27Ac) and ATAC-seq revealed over 60,000 candidate *cis*-regulatory elements (cCREs) active in GCs, e.g. upstream of *FOXL2*. Chromatin interaction mapping using Hi-C revealed GC-specific chromatin loops, of which 292 were differential between cumulus and mural GCs. Additionally, UMI-4C for *FOXL2* identified GC-specific promoter-CRE interactions.

Conclusion: This first comprehensive regulatory map of human GCs provides new insights into gene regulation of the ovarian somatic compartment, advancing our understanding of GC-related female infertility, such as POI.

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P02.013.C Exploring the suitability of optimised, "Goldilocks" pan-ancestry carrier screening tests in endogamous and bottlenecked populations

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Background: Expanded reproductive genetic carrier screening ("carrier screening") assesses reproductive risk for autosomal recessive and X-linked conditions and may reduce the incidence of screened conditions in exogamous, mixed ancestry, and specific racial/ethnic/ancestry groups. However, its effectiveness in "edge cases", such as endogamous or highly bottlenecked populations, remains unclear. We developed "Goldilocks" carrier screening panels to optimize detection of heterozygotes and carrier couples across diverse ancestry groups at minimal cost. This study evaluates the performance of these Goldilocks panels in edge cases.

Material and methods: We analysed gene carrier rates for 1,350 genes using aggregated allele frequency data from exome/genome sequencing (ES/GS). For endogamous groups, we used Ashkenazi Jewish and Finnish AF data from gnomAD v4.1.0 (joint ES/GS). For bottlenecked groups, we analyzed Amish data from 7,221 Amish ES samples (including all Amish AF data in gnomAD v4.1.0). Goldilocks panels were assessed by evaluating the proportion of heterozygotes and carrier couples identified.

Results: Goldilocks panels performed well in endogamous and bottlenecked populations. A Goldilocks panel of only 200 genes was sufficient to achieve 99% detection of heterozygous individuals and carrier couples, provided those groups were adequately represented in the dataset.

Conclusion: Theoretical pan-ancestry "Goldilocks" carrier screening panels remain clinically effective in reproductive edge cases, provided frequency data adequately represents the target populations. This highlights the importance of population representation in carrier screening panel design.

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P02.014.D SantPauCarriers: a pilot study for the implementation of preconceptional carrier screening

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Background: Severe genetic diseases entail significant emotional and financial burdens on families and the public healthcare system. Preconception carrier screening identifies at-risk couples for preventive action. In Spain, such screenings are limited to private settings, particularly in assisted reproduction, leaving the public healthcare system without equivalent options and creating inequities in access.

Material and methods: We conducted a pilot study to implement population-wide preconception carrier screening in primary care. A next-generation sequencing (NGS) panel including 350 genes associated with 450 recessive genetic disorders was designed together with complementary tests for undetectable mechanisms of pathogenicity. Of 518 contacted women, 152 met the criteria (18–38 years, male partner, and reproductive plans). Male partners (66) were tested only if the women were carriers. The process included genetic counseling, blood collection, DNA analysis, and pre- and post-test surveys to assess participants' experiences.

Results: Expression of interest was obtained from 85% of the contacted population (441/518). Among the final studied group, the carrier rate was 62% (135/218), with 4% of couples identified as high-risk (6/152). Survey analysis revealed that participants with lower anxiety levels reported higher perceived control ($p = 0.003$), as well as a positive correlation between participants' educational level and their understanding of the scientific basis of the project ($p = 0.018$).

Conclusion: This pilot study has identified bottlenecks and opportunities for improvement in the implementation of a population-wide carrier screening. It highlights the acceptance and feasibility of integrating preconception carrier screening into public healthcare as a valuable tool for informed reproductive decision-making.

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P02.015.A Investigating the regulatory upstream factors of the PTPN1 gene associated with insulin resistance in women with polycystic ovary syndrome

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Background: Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder affecting many reproductive-age women, with insulin resistance seen in 50–70% of cases. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression, including Protein Tyrosine Phosphatase Non-Receptor Type 1 (PTPN1), crucial for insulin signaling. Alterations in PTPN1 expression may lead to insulin resistance. This article explores miRNAs as upstream regulators of PTPN1 in relation to insulin resistance in PCOS.

Material and methods: Bioinformatics analysis identified miRNAs targeting PTPN1. The STRING database predicted interactions of key genes with PTPN1, while three databases (miRDB, mirDIP, and miRWalk) facilitated an in-silico search for relevant miRNAs. High-scoring miRNAs were examined using TargetScan and miRBase for expression levels. Ovarian granulosa cells were collected from 36 women with PCOS and 36 age-matched controls, measuring PTPN1 and circulating miRNAs using quantitative polymerase chain reaction (qPCR).

Results: In PCOS patients, miR-22-3p and miR-6511a-3p levels were significantly higher in granulosa cells and follicular fluid compared to normal subjects. Conversely, miR-6795-5p, miR-129-2-3p, and miR-506-3p were downregulated. Additionally, PTPN1 gene expression was notably higher in PCOS patients. Bioinformatics analysis indicated that PTPN1 is a downstream target of miR-506.

Conclusion: In PCOS patients, increased levels of miR-22-3p and miR-6511a-3p were noted, while downregulated miRNAs may promote insulin resistance by targeting PTPN1, suggesting potential therapeutic targets and non-invasive biomarkers for diagnosis.

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P02.016.B Comparative efficacy of cfDNA and aCGH in detecting chromosomal aberrations post-miscarriage

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Background: Miscarriages affect 10–25% of pregnancies, 1–5% of couples experiencing recurrent losses. Chromosomal abnormalities are a leading cause, yet traditional genetic testing on products of conception (POC) has limitations, including the need for intact tissue and absence of maternal DNA contamination. This study compares cell-free DNA (cfDNA)-based testing with array comparative genomic hybridization (aCGH) in diagnosing miscarriage etiology.

Material &methods: In our prospective multicenter study, we recruited 78 patients experiencing pregnancy loss between July 2023 and July 2024. Maternal blood samples collected before curettage underwent cfDNA low-depth genome sequencing

through a standard NIPT workflow, while POC samples were analyzed via aCGH post-curettage.

Results: Of the 78 participants, 13 were excluded due to missing POC samples. Among the remaining 65, 18 yielded results from only one technique (16 cfDNA, 2 aCGH), and both failed in two cases. Maternal contamination primarily caused aCGH failures. Concordance between cfDNA and aCGH was observed in 82% (37/45). Notably, cfDNA detected 6 unique chromosomal aberrations and identified 13 out of 19 aberrations found by aCGH (aneuploidies only). Sensitivity and specificity of cfDNA testing were 68.4% and 92%, respectively.

Conclusion: Our findings highlight cfDNA's potential as a robust alternative to traditional POC testing, offering advantages in scenarios where maternal contamination or sample availability/integrity is problematic. Analytic thresholds to call chromosomal aberrations from cfDNA still have to be refined to the context of pregnancy loss. These results underscore the value of incorporating cfDNA-based tests into routine miscarriage investigations, including the possibility to test patients that do not require curettage.

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P02.017.C Determining the pre- and post-fertilization origins of aneuploidy: a new paradigm in assisted reproduction to substantially improve embryo selection

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Background: Preimplantation genetic testing for aneuploidy (PGT-A) is widely used in in vitro fertilization for embryo selection. However, large clinical trials have questioned its efficacy, as current PGT-A may deselect viable embryos. Recent findings indicate that mosaic embryos, i.e. embryos containing both euploid and aneuploid cells, can lead to healthy live births. We evaluated the clinical utility of PGT for aneuploidy origin (PGT-AO), which determines the meiotic/mitotic origin of aneuploidies.

Material and methods: In a nationwide non-selection study, we retrospectively analyzed the genomes of 5,975 embryos sequenced for PGT for monogenic disorders (PGT-M) using either genotyping-by-sequencing (n = 2,493) or whole-genome sequencing (n = 3,482). Genome haplarithmisis determined the meiotic/mitotic origin of aneuploidies and their mosaicism. The aberrations were correlated with recorded clinical outcomes.

Results: Among 946 transferred embryos, 253 (26.7%) resulted in live birth, while 693 (73.3%) failed to reach live birth, including implantation failure (n = 546, 78.8%), early embryonic arrest (n = 104, 15.0%) and pregnancy loss (n = 43, 6.2%). None of the embryo with a meiotic trisomy resulted in live birth, while 17.0% of embryos with a mosaic mitotic trisomy did. Furthermore, only 6.8% of monosomic embryos resulted in live birth. Remarkably, different ovarian stimulation protocols influenced transfer outcomes and aneuploidy rates.

Conclusion: Embryos with meiotic trisomies and most monosomies should be considered non-viable, while those with mitotic trisomies can result in healthy live births. PGT-AO in combination with sequencing-based PGT-M will avoid unnecessary embryo transfer. PGT-AO will potentially make a paradigm-shift in embryo selection within a PGT-M and PGT for structural rearrangements (PGT-SR) context.

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P02.018.D *XIST* and *FAM9A* methylation patterns as indicators of spermatogenesis status in patients with Klinefelter syndrome

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Background: In Klinefelter syndrome (KS), small testes phenotype happens due to a diminished germ cell number with two types of testicular histology, comprising of Sertoli cell-only tubules and those with focal spermatogenesis. Recently it was found that mature Sertoli cells in germ cell-containing tubules of KS patients lack the expression of XIST. Another X-chromosome related gene, FAM9A, was reported to be up-regulated in KS testes. Hereof we aimed to check whether the methylation status of these two X-related genes could be related to the presence of germ cells in KS testes.

Material and methods: Patients with azoospermia were recruited and cytogenetically screened at the University Hospital Centre Zagreb. DNA was isolated from blood and testicular tissue of 6 KS patients showing either no germ cells or focal spermatogenesis, and from 3 patients with full spermatogenesis (control). After bisulfite conversion, pyrosequencing was performed to detect the percentage of XIST and FAM9A methylation.

Results: Blood samples of Klinefelter syndrome patients showed a 50% decrease in XIST methylation, whereas in males with full spermatogenesis the XIST gene was ~100% methylated. XIST methylation in testes without germ cells was the lowest (22%) but not significantly different from control or testes with germ cells (28% and 36%, respectively). FAM9A was strongly methylated (~90%) in all samples.

Conclusion: XIST methylation status in testicular tissue indicates the immaturity of KS patient's testes, but is not a reliable marker of focal spermatogenesis. No clear differences in the inactivation of FAM9A gene in KS patients and controls were found.

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P02.019.A Genetic causes of familial hypospadias in Qatar: insights from Sidra Hypospadia Biobank in Qatar and whole genome sequencing analysis

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Background: Hypospadias is a common congenital anomaly in male infants and involves underdevelopment of the ventral aspect of the penile shaft. Genetic factors significantly contribute to the etiology of hypospadias. Identifying genetic contributors, especially in understudied populations, can enhance our understanding and inform targeted interventions.

Material and methods: This study utilized a structured biobank to collect and analyze biospecimens from hypospadias cases and their relatives. Whole genome sequencing (WGS) was performed on 27 subjects from seven families to identify potential genetic causes. Bioinformatics tools, including GEMINI, were used to assess the segregation of single genetic variants (SNVs) with the condition within families.

Results: Our SNV analysis identified at least four likely pathogenic variants, in four of the index patients, in genes that were not linked to hypospadias before. These variants were located in the *LHFP*, *EIF2B5*, *INO80*, and *ACADVL* genes and segregated with the hypospadias phenotype. Also, we have identified variants of uncertain significance in known hypospadias genes (DNAH12 and HOXB6) and other genes (*COL6A3)*. Two families did not reveal any potential causative variants and would require further genetic investigation, such as copy number variant (CNV) analysis. Future functional work will be necessary to establish causality between the novel SNVs identified and the hypospadias phenotype.

Conclusion: Our findings underscore the value of disease biobanking and genetic investigations in uncovering the pathogenesis of congenital conditions like hypospadias. Our findings provide a new avenue to functionally assess the identified variant(s) in relation to disease, and to better understanding disease phathophysiology.

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P02.020.B Low-level mosaicism on sex chromosomes and carrier's sex in families with pregnancy pathologies

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Background: Low-level mosaicism (LLM) on sex chromosomes may remain undiagnosed and cause unpredictable reproductive disorders. Goal**:** Analysis of the association of low-level sex chromosome mosaicism in both male and female family members with tubal pregnancy (TP) and recurrent pregnancy loss (RPL).

Material and methods: Karyotypes of 4418 individuals with reproductive disorders were analyzed, cytogenetic abnormalities were identified in 809, in 415 women and in 394 men. Patients with detected mosaicism using GTG-banding underwent additional examination by FISH on uncultured peripheral blood lymphocytes.

Results: LLM up to 10% on sex chromosomes, for example, 45, X/46, XX, 45,X/47,XXX/46,XX, 47,XXY/46,XY, was found in 78 patients (9.6%). In women, it was determined 3 times more often than in men, 14.2% vs 4.8%, ($p < 0.001$). Among patients with chromosomal abnormalities, individuals from families with TP accounted for 2.1% ($n = 17$). The ratio of women (34.0 ± 5.1) and men (33.0 ± 4.3) was 1.5:1 ($p = 0.46$). Individuals from families with RPL were 5.0% ($n = 40$). The ratio of women (32.1 ± 5.0) and men (33.3 ± 9.5) is 1.5:1 ($p = 0.198$). LLM on sex chromosomes in both women and men increases the risk of TP by 7.6 times (OR = 7.59, 95% CI 1.1–52.3, $p < 0.05$), and RPL by 6 times (OR = 5.96, 95% CI 1.0–35.6, $p < 0.05$).

Conclusion: Low-level mosaicism on sex chromosomes up to 10% increases the risk of tubal pregnancy and habitual miscarriage regardless of the sex of the potential parent-carrier of the chromosomal abnormalities.

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P02.021.C Optimizing non-invasive Preimplantation Genetic Testing (niPGT-A): a comparative analysis of sample collection on Day 5 vs. Day 6

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Background: The selection of euploid embryos is a widespread practice in assisted reproduction (AR) that facilitates achieving pregnancy in a shorter time. Obtaining this selection through a completely non-invasive method represents a significant advancement. Secure Select is a new non-invasive Preimplantation Genetic Test for aneuploidies (niPGT-A) that enables embryo selection.To optimize the performance of Secure Select, it is essential to establish a proper protocol for the collection of culture medium. In this study, we aim to determine the optimal day for culture medium collection.

Material and methods: A total of 219 AR cycles were included, and 599 culture media samples from blastocyst-stage embryos were processed. The NICS kit from Yikon Genomics was used on the Illumina NGS platform (Miniseq). Analysis was performed using the Yikon Genomics ChromGo platform.

Results: The results show that the amplification failure (AF) rate is 21.05% on day 5 compared to 4.61% on day 6. This difference is statistically significant, $p < 0.001$.

Conclusion: The findings indicate that the optimal day for culture medium collection is day 6. These data demonstrate that the Secure Select technique is suitable for embryo selection in a completely non-invasive manner.

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P02.022.D Benefit of the CATSPER2 gene testing in infertile couples

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Background: The protein encoded by the CATSPER2 gene is part of an ion channel that regulates the entry of calcium ions into the sperm flagellum. Increase in the concentration of Ca^{2+} ions initiates sperm hyperactivation, which is required for the zona pellucida penetration and successful oocyte fertilization. Inactivation of the CATSPER2 gene disrupts the hyperactivation process and it has been repeatedly reported as a cause of male infertility. Affected men may have normal spermiogram, therefore the actual cause of infertility remains unrevealed.

Material and methods: The CATSPER2 gene has been examined in our laboratory since 2021 as a part of the preconception carrier screening for recessive monogenic diseases (targeting 103 genes). The test is performed using NGS by analysing both entire coding sequences and copy number variations. A cohort of 77 infertile couples underwent this examination before or during infertility treatment.

Results: A homozygous deletion of CATSPER2 was found in 2 out of 77 (2.5 %) men from the infertile cohort. In both cases, the men were found to have normo-/teratospermia and no obvious cause of infertility was revealed in their female partners. In one of the patients, a homozygous loss of the adjacent STRC gene was detected, demonstrating the genetic causality of his hearing impairment.

Conclusion: Genetic analysis of CATSPER2 can reveal the cause of (male) infertility in a significant proportion of infertile couples and thus help them start an effective treatment faster. Therefore, it would be beneficial to include CATSPER2 in routine genetic testing performed in infertile couples.

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P02.023.A *CHD7* somatic mosaicism in unaffected parents can lead to underestimation of recurrence risk

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Background: CHARGE syndrome (CS) is a rare genetic condition affecting approximately 1 in 12.000–15.000 live births. In recent years an increasing number of cases with germline mosaicism has been reported. We report a female newborn with complex malformations, including four major and at least one minor clinical criteria for CS, born from healthy parents with two healthy children.

Methods: Trio genome sequencing from peripheral blood DNA was performed.

Results: We identified the heterozygous loss of function variant NM_017780.4:c.4953dup;p.(Asn1652Ter) in *CHD7*, which was also present in 10% of paternal reads. Reverse phenotyping of the father revealed mild facial dysmorphism and dysplastic ears, consistent with somatic mosaicism.

Conclusion: This case highlights the importance of thorough phenotyping, genetic counselling and genetic testing of parents, even if they seem to be unaffected. This applies in particular when they wish to have more children since this decision is largely influenced by our assessment regarding the risk of recurrence. Confirmation of a significant recurrence risk through analysis of different paternal tissues including sperm is currently underway.

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P02.024.B Evaluating the predictive ability of polygenic risk score for ntrahepatic Cholestasis of Pregnancy in the Estonian Biobank

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Background: Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder in late pregnancy, characterized by elevated liver enzymes and severe itching. It poses significant risks to fetal health, including stillbirth and preterm birth. Genetic factors contribute to ICP susceptibility, highlighting the potential of polygenic risk scores (PRS) in predicting ICP risk.

Material and methods: Using genome-wide association study (GWAS) summary statistics (1,138 cases, 153,642 controls; Dixon et al., 2022), we calculated PRS for ICP for Estonian Biobank participants, including 1,152 ICP cases and 47,775 controls with delivery-related diagnosis codes but no ICP. Logistic regression, adjusted for birth year and the first 10 genetic principal components (PCs), assessed PRS-ICP associations. Validation was conducted in HUNT study (71 cases, 10,723 controls). A time-to-event analysis on 138 ICP pregnancies and 10,073 controls estimated the hazard of ICP onset using a Cox model adjusted for age at delivery, birth year, and first 10 PCs.

Results: ICP prevalence in Estonian Biobank increased with PRS, from 0.9% in the lowest decile to 6.1% in the highest. The odds ratio (OR) for the highest PRS decile was 3.3 (95CI 2.7–3.9, $p = 1.4 \times 10\text{^-}35$) compared to middle groups, and 1.9 (95CI 1.8–2.0, $p = 1.4 \times 10\text{^-}91$) per PRS standard deviation (SD) increase. The association was confirmed in HUNT (OR 1.7, 95CI 1.3–2.1, $p = 2.8 \times 10\text{^-}5$). Time-to-event analysis showed a hazard ratio (HR) of 2.5 (95CI 2.1–2.9, $p = 1.9 \times 10\text{^-}23$) per PRS SD increase.

Conclusion: PRS is a valuable tool for predicting ICP risk and identifying high-risk women for enhanced monitoring strategies to improve maternal and fetal outcomes.

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P02.025.C A gain-of-function NANOS1 RBP variant alters early human germ cell development by redirecting signalling pathways

Matisa Alla[1], ***Bellary Lakshmi***[1], *Maciej Jerzy Śmiałek*[1,2], *Marcin Piotr Sajek*[1,3,4], *Krystyna Kwiatkowska*[1], *Żaneta Lemańska*[1], *Dorota Klaudyna Kiżewsk*[5], *Małgorzata Borowiak*[5], *Wojciech Juzwa*[6], *Kamila Kusz-Zamelczyk*[1], *Alex Rayevsky*[7,8], *Jadwiga Jaruzelska*[1]

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Background: NANOS is an RNA-binding protein that is specifically expressed in germ cells across species, where it plays a crucial role in inhibiting somatic fate. NANOS1 with p.[Pro34Thr; Ser78del] mutation is linked to absence of germ cells in patients. Our objective was comparing the RNP interactomes of wt- and v-NANOS1 in earliest phases of human PGC specification/development to identify pathways important for germ cell determination.

Material and methods: A PiggyBac-based, transgenic, in-vitro inducible NANOS1 expression system was established to investigate human PGC specification/early development. Human embryonic stem cell (hESC) line engineered with a NANOS3-tdTomato reporter for PGC specification enabled FACS sorting. eCLIP combined with RNA-seq/DGE analysis was performed to identify NANOS1-bound-mRNAs across the stages of pre-mesendodermal cells, and early PGCs. NANOS1-bound-proteins were identified and quantified via co-IP followed by eight-plex tandem mass tag (TMT) labelling.

Results: The v-NANOS1 gain-of-function mutation initially downregulates canonical WNT signalling, vital for pre-mesenodermal precursor induction toward PGCs. During PGC specification, v-NANOS1 fails to repress upregulated canonical WNT and TGF-ß targets, leading to increased EMT and WNT activity. This shift is reflected by higher late mesoderm marker expression and lower early germ cell marker expression, indicating a transition toward a somatic cell profile. Proteomic analysis between pre-mesenodermal and PGC stages revealed an enrichment of 17 proteins, including 10 centrosomal proteins, in the v-NANOS1 interactome. Validation of these findings is ongoing.

Conclusion: We anticipate that through upregulating mesodermal markers, v-NANOS1 acquires a gain of function. This asserts cells towards differentiation of mesodermal derivatives rather than PGCs.

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P02.026.D Subpopulations of human follicular fluid extracellular vesicles differentially influence KGN granulosa cell function

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Background: Extracellular vesicles (EVs) are lipid-bilayered particles that play critical roles in intercellular communication. The functional differences between EV subpopulations within the human ovarian follicle remain poorly understood. In this study, we investigated the effects of small (SEV) and large EVs (LEV) isolated from preovulatory follicular fluid (FF) of in vitro fertilization (IVF) patients on mRNA and non-coding RNA expression in granulosa-like KGN cells.

Material and methods: Ethical approval was obtained from the University of Tartu Research Ethics Committee. Written informed consent was obtained from study participants. FF EV subtypes were isolated using size exclusion chromatography and tangential flow filtration. KGN cells were treated with 108/ml LEVs or 109/ml SEVs for 24 hours. RNA-sequencing and small RNA-sequencing libraries were prepared, sequenced, analyzed for differential expression using DESeq2, followed by pathway enrichment analysis using ShinyGo and miEAA.

Results: LEV treatment altered the expression of five genes: KIRREL3, CCBE1, VCAM1, C1S, and SOD2. SEV treatment triggered significant transcriptomic changes, with 692 genes upregulated and 904 downregulated. Downregulated genes were linked to cell cycle and Rho GTPase signaling, while upregulated genes were associated with extracellular matrix remodeling and MET signaling. SEV treatment also altered miRNA expression in KGN cells, upregulating 42 and downregulating 27 miRNAs compared to LEVs. Upregulated miRNAs were related to transcription, receptor tyrosine kinase signaling, and TGF-β signaling.

Conclusion: These findings highlight the functional distinctions between EV subpopulations in human FF and underscore the importance of considering EV heterogeneity in the studies of intercellular communication.

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P02.027.A RNA sequencing of uterine leiomyoma tumors and control myometrium detects relationships with cancer genes and pathways

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Background: Uterine leiomyomata (UL, benign tumors of uterus) affect 77% of U.S. women by menopause and account for up to $34 billion in annual healthcare costs. Fibroid heritability ranges from 20–69%. Although studies have evaluated fibroid somatic, few studies have utilized RNA sequencing (RNAseq). Here we conduct RNAseq analyses and annotate associations at known GWAS loci.

Materials and methods: We conducted RNAseq on 35 UL tumors and 53 non-fibroid myometrium from women ages 18–50 undergoing hysterectomy with no cancer history, cancer treatment, or infectious diseases. DESeq2 modeled differential expression between fibroid and myometrial tissue controlling for race. Functional enrichment analysis was performed using GSEA using hallmark and oncogene gene sets.

Results: There were 716 genes with significant differential expression (false discovery rate [FDR]-adjusted p[P_{adj}] < 0.05, log2 fold change > |2|). The strongest association was observed at special AT-rich sequence-binding protein 2 ($p_{adj} = 4.68 \times 10^{-83}$). We observed significant associations with seven genes associated with UL in the NHGRI-EBI GWAS catalog. From GSEA, 14 gene sets show evidence of upregulation and eight show evidence of downregulation ($p_{adj} < 0.05$). Notable results were upregulated hallmark genes for MYC targets v1, oxidative phosphorylation, and E2F targets and downregulated hallmark gene sets for estrogen

response early that have been implicated in cancer, including ER+ breast cancer.

Conclusion: Using RNAseq we identified multiple genes and pathways associated with UL risk implicating tumorigenesis pathways. We also observed evidence of differential gene expression and several previously published GWAS loci, further supporting biological roles for these loci in UL risk.

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P02.028.B Pregnancy loss genome-wide association study in UK Biobank replicates associations with common variants at *FAF1* and *PARG1*

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Background: Pregnancy losses affect 15% of recognised pregnancies. The largest genome-wide association study (GWAS) on pregnancy loss identified 10 associated loci with sporadic pregnancy loss (Reynoso et al, 2024, medrxiv:2024.03.20.24304624). Few of these loci are replicated primarily due to challenges in defining the phenotypes. This study investigated pregnancy loss susceptibility using multi-modal data from UK Biobank.

Material and methods: In UK Biobank European ancestry women, we selected 44,891 cases with self-report or electronic health record (EHR) of pregnancy loss (ICD10: O03, O02.1, N96, O26.2) and 116,060 controls with live birth and no history of pregnancy loss. We performed a GWAS with REGENIE software tool, which accounts for population structure, for 10,880,079 single nucleotide variants (SNVs) with minor allele frequency (MAF) ≥ 0.006 from the genome-wide imputed data, using a ridge regression-based model. We also examined eight previously associated loci that were available in our dataset.

Results: We identified suggestive (P-value < 10^{-5}, MAF > 0.05) associations at/near genes implicated in pregnancy loss mechanisms, including *DOCK8* (immune response), *TRIM29* (cell differentiation), and *PSMF1* gene (protein turnover). We also replicated two association signals (Bonferroni corrected P-value < 0.00625, 8 tests), at *FAF1* (rs10888690-C, effect allele frequency (EAF)=0.41, OR(95%CI) = 1.023(1.0067–1.039), P-value = 0.0052) and *PRAG1* (rs2920991-A, EAF = 0.50, OR(95%CI) = 1.023(1.0073–1.039), P-value = 0.0040).

Conclusion: We successfully replicated associations at genes involved in cell cycle regulation and cell morphology regulation, using a more stringent control definition of female participants with live birth and no EHR or self-report of pregnancy loss, despite using less than a quarter of the replication dataset sample size from the original study.

C.O.I. Disclosure:

C. Chu: None. **Y. Sharhorodska:** None. **I. Prokopenko:** None.

P02.029.C Methylation risk score modelling indicates potential non-genetic DNA methylation effects associated with endometriosis

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Background: Endometriosis is a chronic gynaecological disease characterised by endometrial-like tissue found external to the uterus. Although compelling evidence supports a genetic contribution to the disease, research on the environmental impact remains limited. DNA methylation (DNAm), influenced by genetic and environmental factors, is a valuable biomarker for understanding how genetic and environmental exposures affect complex diseases. This study aims to develop a methylation risk score (MRS) for endometriosis to improve the detection of DNAm signals linked to the disease and to gain insights into its pathogenesis.

Material and methods: Endometrium tissue samples from 318 controls and 590 endometriosis cases were collected and analysed to obtain endometrial methylation and genotyping information. Samples were first split into several independent test and training cohorts. Various MRS were generated using different models and their endometriosis case-control classification performance was assessed using the area under the receiver operating characteristic curve (AUC).

Results: The proportion of phenotypic variance in endometriosis explained by DNAm and common genetic variants was 18.25% and 23.78%, respectively. The maximum AUC obtained from the best-performing MRS was 0.68, derived from 746 DNAm sites. Additionally, combining MRS with polygenic risk scores (PRS) consistently outperformed PRS and MRS alone in classification performance.

Conclusion: Our results indicate that there are DNAm signals independent of common genetic variants associated with endometriosis, highlighting the potential role of non-genetic mechanisms in endometriosis.

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P02.030.D Higher incidence of rare predicted pathogenic variants in familial spontaneous preterm birth cases suggests possible heritability risk

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Background: The maternal genetic contribution to spontaneous preterm birth (sPTB) remains poorly understood, although rare variants may influence sPTB risk, particularly in familial cases. This study aimed to evaluate the role of rare predicted pathogenic (RPP) variants in familial and sporadic sPTB, hypothesizing that RPP variants are more prevalent in familial cases.

Material and methods: Whole-exome sequencing (WES) was performed on 31 familial sPTB, 59 sporadic sPTB, and 98 controls. We conducted burden analysis of RPP variants in an sPTB gene panel, including 36 previously associated genes, and explored novel genes linked to sPTB. To explore the potential biological impact of identified RPP variants, pathway analysis using the Enrichr tool was conducted.

Results: Burden analysis revealed no significant enrichment of RPP variants in the sPTB gene panel across combined sPTB, familial, or sporadic groups ($P = 0.406$, $P = 0.309$, $P = 0.417$). However, familial cases had a significantly higher frequency of RPP variants compared to sporadic cases ($P = 0.036$). Pathway analysis identified pyrimidine metabolism as the most significantly enriched pathway ($P = 4.75 \times 10^{-4}$).

Conclusion: Although rare variants in known sPTB-associated genes do not significantly contribute to familial sPTB, familial cases exhibit a higher frequency of RPP variants across the entire exome, suggesting a potential genetic contribution beyond the sPTB gene panel. Pyrimidine metabolism is identified as a potential pathway for further investigation.

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P02.031.A Endometrial fluid as a surrogate tissue for identifying methylation-based biomarkers of breast bancer risk

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Background: Effective tools for risk stratification in Breast Cancer (BC) prevention are critically needed. Recent studies suggest that DNA methylation (DNAm) profiles and methylation Quantitative Trait Loci (mQTL) from cervical cytology samples could serve as surrogate hormone-responsive tissues for monitoring preventive strategies. Given that endometrial fluid represents a liquid biopsy derived from the hormone-responsive endometrium, our study aims to explore its potential as a surrogate tissue for identifying methylation-based biomarkers of BC.

Material and methods: Genotype and methylation data were generated from 145 endometrial fluid DNA samples using the Illumina GSA and EPIC arrays, respectively. Following quality control, the (epi)genomic datasets were combined with TensorQTL and corrected by endometriosis case or control status, age, menstrual cycle phase, and EPIC array batch to identify endometrial fluid mQTLs. These were then integrated with publicly available BC Genome-Wide Association Study (GWAS) summary statistics (GCST004988) using Summary data-based Mendelian Randomization (SMR).

Results: By integrating the 1,131,438 endometrial fluid mQTLs with BC GWAS data, we identified 30 CpG sites showing pleiotropic associations with the disease. Several of these CpG sites were located within or near genes known to be differentially expressed in BC (*L3MBTL3, TNNT3, EBF1, ADCY3*) or associated with aberrant methylation patterns reported in this type of cancer (*ISYNA1, GATAD2A*).

Conclusion: These findings highlight the potential of endometrial fluid as a surrogate tissue for identifying methylation-based biomarkers associated with BC risk, paving the way for innovative strategies in BC prevention and risk assessment.

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P02.032.B Investigation of meiotic recombination as a potential biomarker for embryo viability and pregnancy outcomes

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Background: The relationship between recombination and aneuploidy is well established. Our recent novel findings also show 17% of triploid embryos lack genome-wide recombination. However, despite its important role in meiosis, little is known about the impact of recombination on pregnancy outcomes in euploid embryos. This study examines whether recombination rates correlate with pregnancy outcome in putatively fertile couples undergoing IVF.

Material and methods: This retrospective multi-centre cohort study analysed recombination rates in 82 couples undergoing 94 IVF cycles with preimplantation genetic testing by SNP-array for a total of 165 transferred euploid embryos. Recombination events were assessed using a haplotyping-based algorithm, and statistical models were used to examine relationships with pregnancy outcomes and embryo morphology.

Results: On average, 33 maternal (SD:8.84) and 19.8 (SD:5.14) paternal recombination events were detected per embryo. No correlation was found between recombination and maternal age ($p = 0.9$). Recombination rates showed variation within families (maternal = 7.33(0–16.9); paternal = 4.29(0–12.02) and cycles (4.9). Smaller chromosomes exhibited statistically higher recombination events per Mb ($p < 2.2e\text{-}16$) but were also more prone to recombination absence, especially if maternal. No correlation was found between recombination and embryo morphology ($p = 0.1$). Recombination rates of transferred embryos showed no association with pregnancy outcomes (implantation: maternal p-value = 0.8-paternal p-value = 0.3; miscarriage: maternal p-value = 0.5-paternal p-value = 0.8; live birth:maternal p-value = 0.6-paternal p-value = 0.4).

Conclusion: This study provides insight into the role of recombination in embryo and oocyte health. While minimal recombination variability was observed in euploid embryos from fertile couples, further research is needed to understand its impact on meiotic integrity and reproductive success in infertility cycles.

C.O.I. Disclosure:

L. Picchetta: A. Employment (full or part-time); Modest; Juno Genetics. **R. Cavagnola:** A. Employment (full or part-time); Modest; Juno Genetics. **C. Ottolini:** A. Employment (full or part-time); Modest; Juno Genetics. **E. Paraboschi:** A. Employment (full or part-time); Modest; Juno Genetics. **F. Mulas:** A. Employment (full or part-time); Modest; Juno Genetics. **A. Haj Ali:** A. Employment (full or part-time); Modest; Juno Genetics. **D. Babariya:** A. Employment (full or part-time); Modest; Juno Genetics. **G. Clark:** A. Employment (full or part-time); Modest; Juno Genetics. **D. Wells:** A. Employment (full or part-time); Modest; Juno Genetics. **A. Capalbo:** A. Employment (full or part-time); Modest; Juno Genetics.

P02.033.C Genomics of idiopathic hypogonadotropic hypogonadism in the Maltese islands

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Background: Idiopathic Hypogonadotropic Hypogonadism (IHH) is a rare and genetically heterogenous condition defined by impaired gonadal function due to insufficient or absent pulsatile secretion of gonadotropin-releasing hormone from the hypothalamus and/or gonadotropins from the anterior pituitary, often presenting with absent or incomplete sexual development and infertility by adulthood. Our objective was to characterise a cohort of 17 probands.

Material and methods: Targeted gene panel and whole exome/genome sequencing data were generated using Illumina. Bioinformatic analysis and in silico predictions were implemented to shortlist variants. Calcium mobilisation and IP1 assays were used to characterise novel *KISS1R* and *GNRHR* variants.

Results: We identified causative genetic variants in eight probands and identified potential leads in four. The findings were: (i) *FGFR1* p.Y132C in a man with cryptorchidism and micropenis, (ii) a woman with secondary amenorrhoea with a heterozygous *GNRHR* p.Q106R variant, which was also present in her (iii) hyposmic father, who had a novel *CCDC141* variant, (iv) identification of a novel *CHD7* stop variant in an anosmic man displaying two CHARGE features alongside cryptorchidism, (v) a woman with compound heterozygosity for *GNRHR* p.Q106R and a novel *GNRHR* frameshift, (vi) a woman with intellectual disability with a *SOX2* variant, and (vii) a novel *KISS1R* deletion in two brothers. Cases with potential leads included: a woman with secondary amenorrhea with accumulated heterozygous kisspeptin variants and 3 men with autosomal dominant *SOX10, SEMA3A* and *SPRY4* variants.

Conclusion: Findings show that novel *KISS1R* and *GNRHR* variants are non-functional and highlight the genetic complexity and heterogeneity of IHH.

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P02.034.D Long-read sequencing enables PGT-M in de novo 16p11.2 deletion cases with limited family history: a case study

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Background: Patients with a 16p11.2 deletion are characterized by speech and language disorder, motor coordination difficulties, psychiatric conditions, and autistic features. These patients may present with a degree of developmental delay of variable severity. Additional features include obesity, seizures, vertebral anomalies, etc. 16p11.2 consists of a heterozygous ~593-kb recurrent deletion. Once detected, prenatal testing or PGT-M can be offered as a method of prevention of transmission to the next generation. PGT-M requires haplotype construction for linkage analysis, which can be challenging when first-degree family members are unavailable, in cases of de novo pathogenic variants, and when the exact breakpoints of a copy number variant (CNV) are unknown.

Materials and methods: We applied multipurpose long-read Nanopore sequencing and adaptive sampling targeting the 10 Mb spanning the deletion region. Bioinformatic analyses addressed both precise CNV breakpoint mapping and de novo variant haplotype phasing, thereby enabling the usage of PGT-M for those cases.

Results: The patient was a 43-year-old single female carrier of a 16p11.2 deletion who was treated at the PGT clinic. First-degree family members were not available. We identified precise breakpoints of the 16p11.2 deletion in the female carrier and the flanking mutant and wild-type alleles were phased by linked long read haplotype construction.

Conclusion: We demonstrate an application of long-read sequencing technology towards facilitating PGT-M for monogenic disorders in cases where traditional methods do not allow phasing and haplotype construction. This technology enables high accuracy PGT-M for unique cases in which conventional linkage methods cannot differentiate between mutant and wild-type alleles.

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P02.035.A Exploring the proteomic signatures of reproductive ageing in women of South-Asian ancestry

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Background: Earlier Menopause is associated with a higher risk of cardiometabolic, musculoskeletal, and cognitive disorders, and is nearly twice as prevalent in South Asia compared to Western countries. Investigating the role of proteins in its onset could provide valuable insights into actionable targets for post-menopausal health, including identifying mechanisms that contribute to accelerated aging in women after this transition.

Methods: In a South-Asian ancestry cohort with clinical, proteomic and genetic information, we: 1. GWASed age at natural menopause (ANM) (survival analysis, N = 20k women), 2. identified early menopause-predictive plasma proteins based on diverging age-trajectories comparing early to normal menopause (7kSomaScanAssay, N = 5k), 3. employed bidirectional 2-sample Mendelian Randomisation (MR) to orient the causal arrow of the relationship between protein levels and menopause.

Results: We replicated 20/283 loci from the largest ANM GWAS (ReproGen Consortium). We used genetic instruments for 88 early menopause-predictive proteins (pQTLs from our cohort), and genetic instruments for ANM as GWAS signals replicated in either the South-Asian cohort or China Kadoorie Biobank. Bidirectional 2-sample MR identified causal associations in both directions. 75% of these implicated proteins as consequences of the menopause (ANM->protein directionality), with SHGB showing the strongest effect. FSHGB showed the strongest effect in the protein->ANM direction. Both signals were expected due to these proteins' reproductive roles.

Conclusion: Our results shed light on additional biological mechanisms involved in reproductive ageing. We additionally aim to develop proteomic clocks to assess biological ageing in relation to menopause and replicate findings in a European ancestry cohort.

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P02.036.B Relevance of Rare variants of the Subcortical Maternal Complex (SCMC) genes for adverse reproductive outcomes and Beckwith-Wiedemann syndrome

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Background: early embryonic development, before and after embryonic genome activation (EGA) is ruled by Maternal-effect genes (MEGs) coding for transcripts, exclusively expressed in oocytes. Relevant among MEGs are genes of the subcortical maternal complex (SCMC), involved in: a)formation and assembly of meiotic and mitotic spindles, b)reorganization of oocyte and zygote compartments, c)regulation of RNA metabolism, and d) zygote genome activation. A spectrum of reproductive disturbances, ranging from infertility to recurrent miscarriages and altered genomic imprinting in offspring appears related to the functions of SCMC proteins. However, the connection between variants in SCMC and reproductive outcomes remains unclear.

Material and methods: we performed whole exome sequencing (WES) analyses on 150 women from three cohorts: a) infertile women under 35 undergoing assisted reproductive technology (ART) without achieving a live birth (n = 59), b) women with recurrent pregnancy losses (n = 77), and c) healthy women with offspring affected by Beckwith-Wiedemann Syndrome (n = 52). A virtual panel of 86 genes classified as MEGs, was designed, prioritized the variants, and evaluated those with MAF <0.05.

Results: On the first subset of 57 women from the 3 cohorts (cohort 1 = 12, cohort 2 = 23, cohort 3 = 22) we found 223 variants, 30% in genes encoding the SCMC. NLRP2 was represented across all cohorts with the highest variant count (n = 18), while OOEP and PADI6 variants dominated in BWS mothers. Consistent with existing literature, NLRP7 and TLE6 variants were exclusively observed in the infertile cohort.

Conclusion: These preliminary findings indicate an enrichment of variants within SCMC genes, suggesting the need for further studies to validate our results.

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P02.037.C Deconvolution of bulk endometrial tissue identifies cell type specific gene regulation associated with endometriosis

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Background: Human endometrium is a complex tissue that undergoes cyclical changes in structure and function in response to circulating steroid hormones. Traditional expression quantitative trait loci (eQTL) analysis in bulk tissue masks cell-type-specific gene regulation, limiting insights into the influence of genetic regulation on endometrial function in health and disease.

Methods: We investigated the dynamic changes in the cellular and molecular environment of the endometrium using genotype and endometrial RNA-sequencing data from 206 European women with (n = 143) and without (n = 63) endometriosis across different phases of the menstrual cycle. Computational deconvolution methods were used to estimate cell-type proportions and gene expression profiles from bulk endometrium RNA-seq, followed by differential expression and cell-type-specific eQTL analysis.

Results: Cell-type proportions in the endometrium varied across the menstrual cycle and were altered in women with endometriosis. Proportions of luminal and ciliated epithelia were significantly (FDR < 0.05) lower in women with endometriosis during the mid-secretory (MS) phase. Differential expression analysis between women with and without endometriosis in the MS phase identified differentially expressed (DE) genes in different cell-types. These included genes associated with endometrial receptivity. DE genes were enriched in RNA metabolism and biogenesis pathways that can mediate fundamental mechanisms of cell proliferation and migration. Incorporating genetic data we identified cell-type-specific eQTLs in endometrium that will be integrated with endometriosis genome-wide association data to identify target genes influencing endometriosis pathogenesis.

Conclusion: Our findings suggests that cell-type deconvolution can improve our understanding of the role of cell-type-specific gene regulation in endometrium, offering novel insights into its role in fertility and endometriosis.

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P02.039.A Restrictive SNP array based PGT-SR in France: go against incidental findings

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Background: Preimplantation genetic testing (PGT) involves the analysis of DNA from embryos to detect genetic abnormalities, including single-gene diseases (PGT-M), structural rearrangement (PGT-SR) and aneuploidy (PGT-A). In France, PGT is strictly regulated under Article L2131-4 of the Code de la Santé Publique, permitting only the detection of genetic abnormalities present in the parents or immediate ascendants. Consequently, PGT-A is prohibited. PGT-SR relies on fluorescence in situ hybridization (FISH), which is limited by technical and interpretative constraints. This approach contrasts with practices in many other countries using whole-genome amplification technologies, with the potential of uncovering incidental findings.To address these limitations, we aim to implement SNP array based PGT while developing a data filtration method to comply with French legislation.

Material and methods: GenomeStudio software was used and chromosome-specific filters were customized to visualize only the minimal genetic information required for diagnosis. Initial validation of these filters was conducted using postnatal SNP array results from individuals with unbalanced translocations.

Results: Five unbalanced samples were successfully confirmed with our customized filters focused near telomeres and excluding recurrent CNVs. Specific additional filters have been developed to identify pathogenic CNVs and insertions.

Conclusion: Our SNP array filtration method is able to identify chromosomal imbalances of interest, while minimizing the risk of incidental findings, according to the French legislation. Further validation is planned on 60 embryos already diagnosed by FISH based PGT-SR. Our next goal is to adapt this method to PGT-M.

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P02.040.B Natural dizygotic twinning is characterized by low polygenicity and linked to female fertility and infertility

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Background: Natural dizygotic twinning (DZT) results from hyperovulation and is often considered an indicator of female fertility. Paradoxically, DZT is also associated with factors linked to subfertility, such as advanced maternal age. We investigated the association between DZT and female fertility by comparing polygenic scores (PGS) for DZT in mothers of twins and singletons who conceived naturally versus those who conceived after medically assisted reproduction (MAR) and estimated genetic correlations between DZT and other fertility-related traits.

Material and methods: We applied GENESIS and SbayesS to assess polygenicity and calculated PGSs for DZT in 4381 twin mothers and 2,797 controls in the Netherlands Twin Register (NTR) and 1,299 twin mothers and 64,810 controls in the Norwegian Mother, Father, and Child Cohort Study (MoBa) based on the largest meta-analysis of DZT.

Results: DZT is not highly polygenic: ~0.20% of SNPs showed a nonzero effect and 17 SNPs contributed to 17.2% of the total predicted SNP heritability of 7.2%. The PGS for DZT captured 1.60% of the variance in DZT liability. PGSs were higher in mothers of naturally conceived twins compared to those with MAR-conceived twins, especially in mothers who used hormonal ovulation induction. Higher PGSs were associated with a shorted time to pregnancy. Genetic correlations were observed for DZT and anovulatory infertility (-0.698, $p = 5.099 \times 10\text{-}5$), PCOS ($-0.278$, $p = 0.035$), and endometriosis (0.279, $p = 0.003$).

Conclusion: A PGS for DZT is a valuable, under-recognized marker of female fertility, meriting further exploration within fertility research to improve reproductive health insights.

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P02.042.D Identifying genetic markers for predicting TESE success in male infertility

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Background: Nearly 75% of infertile men experience oligozoospermia, ranging from mild sperm reduction to complete azoospermia. For men with non-obstructive azoospermia (NOA), testicular sperm extraction (TESE) remains the best option for sperm retrieval, though success is largely unpredictable. Recently, genes involved in gametogenesis have been recognized as critical for male reproductive function, offering not only a potential genetic diagnosis of infertility but also a possible means to predict the presence of isolated spermatogenesis in the testes.

Material and methods: Sixty patients who previously underwent TESE were analyzed using whole-exome sequencing. A curated panel of 240 NOA-related genes was examined, considering only rare (<5% allele frequency in gnomAD NFE) variants predicted to be pathogenic by bioinformatic tools.

Results: In total, 30 patients (50.0%, 95CI%=37.7–62.3%) were identified as carriers of potentially pathogenic variants. These mutations were identified in 59% of unsuccessful TESE cases versus 44% of successful cases (Fisher test $p = 0.29$, $OR = 1.82$), indicating a trend toward sperm absence in carriers. Burden analysis identified *HFM1* and *ZMYND15* as associated with positive TESE outcomes, while *SOX30* correlated with negative outcomes. Interestingly, *SOX30* is predicted to be involved in the differentiation of male germ cells with post-meiotic arrest, as described in animal models. Principal component analysis (PCA) on mutated samples revealed clustering by sperm retrieval outcome, with a Wilcoxon test confirming a significant difference ($p = 0.006$).

Conclusion: Genetic variants may influence TESE outcomes, with specific genes impacting on success or failure. Identifying these variants may enhance personalized infertility diagnostics and improve TESE success predictions.

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P02.043.A Molecular aspects of preimplantation genetic testing (PGT) for Neurofibromatosis type 1 (NF1) in three large European centers: challenges and pitfalls

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Background: NF1 is a neurocutaneous disorder with complete penetrance and extreme clinical variability. It is one of the most frequently requested autosomal dominant indications for PGT. The NF1 gene is large and has a high mutation rate. A high number of variants occur de novo, are mostly unique and more frequently associated with germline mosaicism. These characteristics can pose difficulties in PGT counseling, protocol set-up and treatment.

Material and methods: A retrospective, observational cohort study on PGT protocols with the indication NF1 was performed in three PGT centers (2004–2022): Maastricht University Medical Center+ (the Netherlands, n = 107), University Hospital Brussels (Belgium, n = 119) and University Hospitals of Strasbourg (France, n = 64).

Results: A PGT protocol was developed for 290 couples with 219 different sequence variants in *NF1* (n = 281 couples), of which 53 are unpublished and absent in variant databases, seven intragenic deletions of ≥1 exon(s), one 17q11.2 microdeletion and one translocation involving *NF1*. Mosaicism was detected in 14 out of 175 sporadic cases (8.0%), respectively 10/169 sporadic cases with a sequence variant (5.9%) and 4/6 sporadic cases with a deletion (66.7%, p-value <0.001). A successful PGT protocol was developed for 283 couples (97.6%) requesting PGT for NF1: 5 couples opted out during set-up and for 2 couples, PGT was technically not possible.

Conclusion: A successful protocol could be developed for the vast majority of the PGT requests for NF1. However, the impact of mosaicism is warranted in reproductive counseling and PGT for NF1, especially in *NF1* deletions of ≥1 exon(s).

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P02.044.B Genetic polymorphisms of coagulation and hormonal factors in Bulgarian patients with endometriosis

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Background: Endometriosis affects 2–10% of women in reproductive age. It is a chronic, inflammatory, estrogen dependent disease, and patients with endometriosis appear to be in a hypercoagulable state. The etiology and pathogenesis of endometriosis remain ambiguous. The objective of our study was to evaluate the impact of the polymorphisms in genes for coagulation factors (FV, FII and PAI-1) and in genes, related to estrogen levels and function (CYP19A1 and ESR1) on endometriosis risk.

Material and methods: We investigated the frequency of seven polymorphisms of these genes in Bulgarian patients with endometriosis (n = 30, average age = 32) and compared it with the control group (internal database with healthy Bulgarian women, n = 221 and/or population data from ensemble genome browser). Chi-square test was used for the determination of statistical significance.

Results: We established higher frequency in patients versus controls for heterozygotes of FII mutation G20210A (8.3% vs 4.5%) and for homozygotes of PAI-1 4G/4G (37.5% vs 23%), not reaching statistical significance. Regarding the polymorphism -351 A > G [XbaI] in ESR1, we found higher frequency among patients compared to controls for G allele (40% vs 31%, p < 0.15) and homozygous G/G genotype (17% vs 9%, p < 0.13). There was no markable difference in the allele or genotype frequencies for CYP19A1 between patients and controls.

Conclusion: Genetic mutations in ESR1 may lead to aberrant gene expression and may be involved in pathogenesis and development of endometriosis. It is worthy to continue this study with more patients and including expression data.

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P02.045.C The contribution of the autoimmune process in the genesis of recurrent pregnancy loss

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Background: Celiac disease (CD) is an autoimmune disease and has been associated with several conditions influencing human reproduction. CD genetic components are *HLA-II* class genes known as *HLA-DQ2* and *DQ8*. The aim of study was to investigate distribution of *HLA-DQ2.5* (*HLA-DQA1*05:01 + HLA-DQB1*02*) and *HLA-DQ8* (*HLA-DQB1*03:02*) genotypes among women with recurrent pregnancy loss (RPL).

Material and methods: SSP-PCR for distribution of *HLA-DQ2.5* and *HLA-DQ8* genotypes. Pooled odds ratio (OR) and prevalence, with 95% confidence intervals were calculated.

Results: Celiac genotypes summarily are statistically significantly more often present in the group of women with RPL compared to control (46.4% vs. 25%, $p < 0.01$), but we did not establish a significant difference in the frequency of high (*DQ2.5+DQ8*) and low risk (*DQ8*) celiac genotypes ($p > 0.05$) separately in women from the RPL group compared to the control group. As for the medium-high risk genotype (*DQ2.5*), statistical calculations showed a significant ($p < 0.01$) increase of the *DQ2.5*-genotype frequency in this group compared to the control, and the OR proves 3 times increases of RPL risk.

Conclusion: An increased risk of RPL is associated with women carrying *DQ2.5*, a genotype predisposing to CD ($p < 0.01$). The OR proves 3 times increases of RPL risk in the presence of the *DQ2.5* genotype in women. Our results indicate the crucial role for the autoimmune process triggered by CD in the genesis of recurrent pregnancy loss.

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P02.046.D 25 years of PGT in Strasbourg

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Background: Preimplantation genetic testing (PGT) was allowed in France in 1994. Strasbourg PGT center is one of the first center that was licensed in 1999. Today, PGT is performed in five authorized centers in France. The French law restricts PGT to inherited monogenic disorders (PGT-M) and structural rearrangements (PGT-SR) with previously and clearly identified abnormality in the future parent or grand-parent. It is strictly restricted to this specific disorder limiting the use of generic technologies. Aneuploidy screening (PGT-A) is thus prohibited.

Material and methods: During 25 years, we had to adapt to many legal or technical challenges and changes. PGT practice and organisation could be improved thanks to evolutions in embryology and genetics. We present key figures from 25 years of PGT in Strasbourg.

Results: We validated more than 400 specific PCR tests for 176 different genetic diseases (including de novo mutations) and more than 300 specific FISH test for several chromosomal rearrangement and sexing (for X-linked diseases). From October 1999 to October 2024, 2909 patients were referred (67% for PGT-M, 31% for PGT-SR and 1% for double indication), 3424 cycles were started leading to 3210 oocyte retrievals and 2740 biopsies. Fresh or frozen embryo transfer could be achieved in 2822 cycles (TE) resulting in 907 clinical pregnancies (32%/TE) and 854 babies born.

Conclusion: Our expertise allows us to manage more patients and indications. We are now working on generic approaches adapted to the restrictive French law in order to decrease individual set up and reduce time to cycle.

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P02.047.A Preimplantation genetic testing: atypical FISH solution for an unusual translocation

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Background: Preimplantation genetic testing (PGT) is a technique used to identify chromosomal or monogenic abnormalities before transfer of embryos created through in vitro fertilization (IVF). In France, PGT can be offered when a patient presents a high risk of transmitting a genetic disease deemed particularly serious and incurable. We report here a complex cytogenetic PGT case in the context of a familial balanced translocation t(1;17) disrupting *BRCA1*, posing a risk of both chromosomal imbalances and/or cancer predisposition.

Material and methods: At the couple's request and following approval by the local ethics committee, all unbalanced embryos and female balanced embryos at risk for *BRCA1*-related cancer were considered non-transferable. Therefore, in addition to detecting imbalances, PGT was required to identify the translocation if present. Fluorescence In Situ Hybridization (FISH) was performed using a combination of five probes. Two probes were located very close on either side of the breakpoint junction on the derivative chromosome 17 to assess the presence or absence of the *BRCA1* disruption. Validation methods confirmed that the spatial proximity of the two probes reliably identified the balanced translocation on interphase nuclei. The other probes were selected to determine imbalances and the sex of the embryo.

Results: Two embryos were analyzed after two IVF cycles. One carried a chromosomal imbalance. The other was a balanced male embryo not carrying the translocation and could be transferred.

Conclusion: This atypical PGT case using enhanced FISH analysis illustrates the ethical and technical challenges that can arise from a balanced familial chromosomal variant.

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P02.048.B A six-year review of cystic fibrosis carrier testing: is it worth it?

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Background: Reproductive testing for many recessive disorders is promoted widely by commercial companies to low-risk populations. Cystic Fibrosis (CF) is the most common recessive disorder for those of Irish ethnicity (1/19 carrier frequency). CF cascade carrier testing (CF-CCT) is offered to individuals with a family history where the genotype is known. The true utility of CF-CCT for family planning is unknown. We reviewed CF-CCT data to evaluate the number of high-risk couples identified and assess the benefit of carrier testing in a high prevalence disorder.

Material and methods: CF carrier tests from 2018–2024 were analysed ($n = 1006$). Carrier tests performed for parents of an affected child, assisted reproduction, or where family history was unknown were excluded ($n = 239$). Data extracted from CF-CCT reports ($n = 767$) included ascertainment (family history), relatedness of proband, and post-test reproductive risk where applicable.

Results: The largest ascertainment for CF-CCT (32.5%;n = 249) was for abnormal newborn screening (NBS) in unaffected children. 27.5% (n = 211) of CF-CCT had a family history, a quarter were first-degree relatives; the most distant were sixth-degree relatives. 190 test reports were couples reports, and testing increased CF reproductive risk for 2.6% couples (n = 5/190). Two of these couples had prenatal genetic testing. The other couples were counselled about reproductive options but their outcomes are unknown.

Conclusion: Broadly implemented CF-CCT shows increased reproductive risk in a small proportion of couples despite being the most highly prevalent recessive disorder in Ireland. Its relatively low yield raises questions about reproductive counselling strategies for disorders with lower prevalence in Ireland.

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P02.049.C Endometriosis (EM) symptoms and extra-oral taste-associated genes (TGs): not only a matter of taste

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Background: EM is a multifactorial gynaecological condition heavily impacting patients' well-being; to date, the mechanisms underlying EM-symptoms variability are unknown. In this context, the role of TGs in complex disorders' etiopathogenesis is emerging; indeed, beyond influencing food choices, they can modulate inflammatory processes. Hence, deepening their effect could provide novel perspectives regarding EM-symptoms development/severity.

Material and methods: A cohort of 110 Caucasian EM patients was enrolled at IRCCS "Burlo-Garofolo" (Italy). Patients underwent a careful clinical examination, comprising EM-symptoms assessment. A list of 23 variants within TGs was extracted from SNP-arrays data. Associations between EM-symptoms presence/severity and TGs were analysed with regression models.

Results: Preliminary results ($p < 0.05$) highlighted the possible impact of TGs in regulating EM-symptoms presence/severity.In particular:- C allele carriers of rs35874116 (*TAS1R2*) displayed a lower risk of dysmenorrhea development (OR = 0.12). Recent findings described that T allele carriers consume more carbohydrates (PMID:39861458); hence, C allele carriers, by consuming fewer sugars, may be protected against dysmenorrhea onset.- A allele carriers of rs224534 (*TRPV1*) had lower risk of post-menstrual pain onset (OR = 0.19). Since a) *TRPV1* encodes a channel regulating noxious stimuli detection, and b) A allele carriers of rs224534 can better respond to acetaminophen (PMID:30908574), this result could open new insights into pain processing and anti-inflammatory drugs response.

Conclusion: This is the first worldwide study investigating TGs in EM. These findings could guide a deeper understanding of EM-symptoms mechanisms, thus allowing the development of personalized strategies to improve patients' management. To further validate these results, expansion of the cohort is ongoing.

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P02.050.D The DNA damage-sensing cGAS-STING pathway is critical in specific telomere Variant Sequence synthesis in men with nonobstructive azoospermia and individuals with differences of sex development

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Background: Increased DNA damage in men with nonobstructive azoospermia (NOA) and individuals with Differences of Sex Development (DSD) was associated with increased telomere length (TL). Autophagy inhibition elongated TL and rescued DNA damage in leukocytes, linking to the Androgen Receptor (AR) and cytoplasmic DNA-sensing cGAS-STING signaling. Recently discovered Telomere Variant Sequences (TVS) bring new focus on genome instability during infertility.

Material and methods: Lymphoblastoid lines (n = 4) and blood leukocytes of NOA groups (abnormal/normal embryogenesis after ICSI; Spermatogonia Arrest (SGA), Sertoli Cells Only (SCO) syndrome), and DSD groups (Swyer, Complete Androgen Insensitivity Syndromes (CAIS) were analyzed with PCR (n = 36) for normal Telomere Sequence (TS: TTAGGG) and TVS (TVS1: TGAGGG; TVS2: GTAGGG; TVS3: TTGGGG; TVS4: TTTGGG; TVS5: TTCGGG), TL with quantitative real-time PCR. Proteins were analyzed with immunoblotting. Unpaired t test was applied for statistics.

Results: Infertile Individuals exhibit TVS in elongated telomeres, linked to telomerase complex protein GAR1 upregulation (Swyer: p = 0,0022; Turner, p = 0,0401; abnormal ICSI group, p = 0,0270, SGA, p = 0,0176). TVS3 was present in of all infertile groups. Inhibitors of autophagy BafilomycinA1 and AR signaling Enzalutamide caused GAR1 upregulation (p = 0,0286), TL extension (p = 0,0085), but increased DNA damage marked by γH2AX on double-strand brakes (p = 0,0286) in DSD cell lines. GAR1-siRNA prevented TVS3 synthesis under Enzalutamide-induced genotoxic stress, which was rescued by Bafilomycin A1. Similarly, in contrast to fertile control, primary leukocytes from DSD patients promoted GAR1 (p = 0,0048) and TVS3 downstream from STING, upon BafA1 exposure.

Conclusion: General molecular mechanisms drive infertility-associated genome instability through AR and autophagy alteration of telomerase complex causing TVS.

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P02.051.A *KIRAA*-genotype as a potential marker recurrent implantation failure after in vitro fertilization

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Background: About 10% of women suffer from recurrent implantation failure (RIF) after in vitro fertilization that is generally described as the absence of pregnancy after the transfer more than four good-quality embryos in a woman under the age of 40. The etiology of RIF remains unclear in almost 50% of cases, with

considerable attention paid to the role of uterine natural killer cells and their KIR receptors. Therefore, the *aim* of the study was to analyze the frequency of *KIR*-genotypes in women with RIF compared to control.

Material and methods: *KIR*-genotyping by PCR-SSP was carried out in the 158 women with RIF and 431 women without reproductive losses.

Results: The frequency and distribution of *KIR*-genotypes in the study groups were analyzed. In women with RIF, a shifted distribution of the frequency of KIR-genotypes was observed, towards the *AA*-genotype, the frequency of which was half of all identified genotypes (50.6%). The distribution and frequency of *KIR*-genotypes in the control group corresponded to the Hardy-Weinberg equilibrium. Statistical analysis revealed a significantly higher frequency of the *KIRAA*-genotype ($\chi 2 = 26.7; p < 0.0001$) in the group of women with RIF compared to the control group of women. If a woman has *KIR-AA*, the risk of repeated failed implantations increases threefold (OR = 2.7).

Conclusion: We consider that the *KIRAA*-genotype is the optimal marker of RIF and we argue that *KIR*-genotyping is a necessary genetic test to determine the risk of embryo rejection by the maternal immune system, which will help to apply the correct medical interventions and save the pregnancy.

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P02.052.B Examination of the link between mitochondrial dysfunction and common endocrine disorders in female reproductive health among Hungarian patients

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Background: Mitochondrial dysfunction is identified as a significant pathogenic factor in the development of prevalent reproductive endocrine disorders. This study sought to assess the relationship between female reproductive hormone levels and multiorgan involvement, their effects on plasma GDF-15 levels, and the presence of mtDNA deletions.

Material and methods: The current study involved 81 female patients who were treated for insulin resistance (IR) alone, as well as those with IR combined with polycystic ovary syndrome (PCOS), and IR with premature ovarian insufficiency (POI), alongside 41 healthy female controls.

Results: There is a significant inverse correlation between GDF-15 levels and TSH values in the overall cohort ($p = 0.023$). However, in the groups with and without mtDNA deletion, only a trend is observed. Patients with mtDNA deletion exhibited lower AMH levels compared to those without it. In the IR+PCOS subgroup, total testosterone levels rise alongside increasing GDF-15 levels. Conversely, in the IR-only subgroup, serum estradiol and testosterone levels tend to decrease as GDF-15 levels rise. The high number of affected organ systems in our group with mtDNA deletion is believed to result from more severe mitochondrial dysfunction.

Conclusion: These findings indicate that the integrity of mtDNA may be vital for sustaining normal ovarian function and hormone production. The multiorgan involvement observed in patients with mtDNA deletions highlights the systemic effects of mitochondrial dysfunction, suggesting that it could influence not only reproductive functions but also have wider implications for overall health.

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P02.053.C Partial AZFc deletions in Moroccan patients with idiopathic infertility

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Background: Infertility affects 10% to 15% of couples trying to conceive, with a male factor identified in nearly half of the cases. The most common molecular abnormality causing male infertility is microdeletion of the long arm of the Y chromosome (MCY). These deletions impact the Azoospermia Factor (AZF), a group of genes involved in spermatogenesis, spread across three distinct regions: AZFa, AZFb, and AZFc.This study aimed to evaluate the prevalence of AZF microdeletions in infertile men from Morocco.

Material and methods: We present a series of 30 infertile Moroccan patients with severe oligospermia (n = 10) or azoospermia (n = 20), who underwent clinical and paraclinical investigations as well as molecular analysis of the AZF regions using the MLPA technique. In parallel, 20 normozoospermic men were analyzed as controls.

Results: Partial AZFc microdeletions were identified in four patients (4/30). However, no AZFa or AZFb deletions were found. The four deletions were observed in two patients with severe oligospermia and two with azoospermia. No deletions were detected in the control group.

Conclusion: According to this study, the frequency of MCY is estimated at 13%. This frequency is comparable to that reported in other studies but higher than previously documented in Moroccan populations. The management strategy for male infertility should systematically include testing for MCY in all patients with azoospermia or severe oligospermia before proceeding with assisted reproductive techniques.

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P02.054.D Expanded carrier screening during pregnancy, reproductive follow-up of 100 consanguineous couples

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Background: Preconception carrier testing (PCT) for autosomal recessive (AR) and/or X-linked conditions can be offered to enhance couples' reproductive decision-making. Consanguineous couples have an increased risk to be a carrier couple for AR conditions. Ideally, testing is performed before conception, but is often requested during pregnancy. Our aim for this study was twofold: 1) to determine diagnostic yield for PCT in consanguineous couples during pregnancy and 2) to gain insight into reproductive decision-making by examining reproductive follow-up.

Subjects and methods: A hundred pregnant consanguineous couples (aged ≥ 18), referred and counselled for PCT by clinical geneticists were included. Exome-based PCT for AR conditions with a large gene panel (>2000 genes) was performed. Diagnostic yield was defined as the percentage of couples who were identified as carrier couples for a condition they were not yet aware of. Reproductive decisions were extracted from patients' medical files.

Results: Diagnostic yield was 25% (25/100). AR conditions couples were carriers of were diseases with a range in severity and variable expressivity; examples were Familial Mediterranean Fever and Joubert Syndrome. Pregnant couples made different decisions after test results, including invasive prenatal testing, continuation and termination of affected pregnancies, postnatal genetic testing and decisions regarding peripartum care. In two cases the PCT resulted in explanations for foetal abnormalities that were not explained by prenatal testing.

Conclusion: These results show that expanded carrier testing during pregnancy in consanguineous couples has a high diagnostic yield. Couples use these PCT results to make different reproductive decisions.

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P02.055.A Age-related trends in embryo eupoidy, aneuploidy, and mosaicism: refining the indications for preimplantation genetic testing (PGT-A)

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Background: Aneuploidy, mosaicism, and euploidy in embryos significantly impact the success rates of in vitro fertilization (IVF). Understanding their distribution across different maternal age groups is crucial for optimizing treatment strategies.

Material and methods: Data on aneuploid, mosaic, and euploid embryos (n = 1010) were collected from patients (n = 451) undergoing Preimplantation Genetic Testing for Aneuploidy (PGT-A) at an IVF clinic. The study included 13 age groups ranging from 20–21 years to 46–47 years. Proportions of each embryo type were calculated for each group.

Results: The proportion of aneuploid embryos increased significantly with maternal age, peaking in older age groups. Euploid embryos predominated until the age of 38 years. In the 38–39 year group, the proportions of euploid and aneuploid embryos were approximately equal, after which the proportion of aneuploid embryos surpassed that of euploid ones. Mosaic embryos remained relatively stable up to the age of 36–37 years, showing a slight increase thereafter but not as pronounced as the rise in aneuploid embryos.

Conclusion: Maternal age critically influences embryo quality, with increasing aneuploidy rates observed with advancing age. We propose revising the recommended age for PGT-A from 35 to 38–39 years based on these findings. This adjustment could optimize patient selection for genetic testing and improve IVF outcomes. Clinicians should consider these trends when advising patients on fertility treatments and associated risks.

The results of PGT-A of embryos from patients of different age groups

AGE GROUPS	ANEUPLOID, %	MOSAIC, %	EUPLOID, %
20–21	22.22	22.22	55.56
24–25	16.67	0	83.33
26–27	0	30.77	69.23
28–29	47.37	10.53	42.11
30–31	40.74	11.11	48.15
32–33	41.11	4.44	54.44
34–35	32.97	5.49	61.54
36–37	41.79	7.96	50.25
38–39	44.16	10.15	45.69
40–41	55.95	10.12	33.93
42–43	73.48	6.82	19.70
44–45	95.83	4.17	0
46–47	83.33	0	16.67

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P02.056.B Chromosomal aberrations and Y Chromosome microdeletions in infertile men: a retrospective study in Eastern Morocco

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Background: Male infertility accounts for 50% of infertile couples. Genetic factors are identified in 15–30% of infertile men. Chromosomal aberrations and AZF microdeletions are the most important genetic causes of male infertility. Our objective is to evaluate, for the first time in the Eastern region of Morocco, the frequency of chromosomal abnormalities and AZF microdeletions in infertile men.

Material and methods: This is a 4-year retrospective study involving 113 patients (70 non-obstructive azoospermic and 43

oligozoospermic men) referred to our medical genetic laboratory at Mohammed VI University Hospital Center of Oujda for genetic exploration. We performed postnatal constitutional karyotyping on venous blood in all patients in our series. Multiplex PCR of the AZF regions of the Y chromosome was performed on 38 patients.

Results: Cytogenetic analysis identified chromosomal aberrations in 13% of cases (14/113), with a frequency of 15.94% in patients with azoospermia and 6.97% in those with severe oligozoospermia. Klinefelter syndrome was the most frequent chromosomal abnormality (n = 9). Structural chromosomal aberrations were observed in four patients: three Robertsonian translocations and one mosaic deletion of the long arm of the Y chromosome. AZF microdeletions were detected in 10.52% of tested cases (4/38), two patients with AZFc microdeletion and two concerning AZFbc. All patients in our series with genetic abnormalities benefited from genetic counseling.

Conclusion: Study of chromosomal aberrations and Y chromosome microdeletions remains a good first-line strategy in the public health system for an accurate diagnosis, appropriate genetic counseling, and improved infertile men management.

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P02.057.C Study of genomic biomarkers associated with preterm birth

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Background: Spontaneous preterm birth (PB), defined as delivery before 22–37 weeks of gestation without medical or surgical induction, is a significant public health challenge with substantial impacts on neonatal mortality and long-term morbidity. Genetic predisposition plays a critical role in PB, with maternal and fetal genetic variants influencing susceptibility through pathways involved in inflammation, immunity, uterine contractility, and cervical remodeling. Fetal genetic variations responsible for crucial organogenesis, affecting placental development and maternal-fetal immune interactions further contribute to PB risk and may lead to adverse health outcomes, especially attention-deficit/hyperactivity disorder and autism spectrum disorders. Additionally, gene-environment interactions, where genetic predisposition amplifies sensitivity to external factors like infection, maternal stress, or smoking, exacerbate the condition's complexity. Advances in genetic studies, such as genome-wide association studies (GWAS), provide valuable insights into PB's underlying mechanisms, paving the way for identifying at-risk individuals and developing targeted interventions. This project aims to identify potential biomarkers for PB on women and preterm infants, to investigate their functional mechanisms, and to understand the early molecular pathways leading to PB.

Material and methods: The study included 25 healthy trios (full-term newborn, mother, and father) and 16 PB trios (mother, father, and preterm newborn). Whole-genome genotyping was performed using the Illumina Infinium Global Diversity 1.8 M Array. Data processing included imputation, annotation, de novo SNPs and GWAS analysis.

Results: Further genotyping and detailed data analysis are currently ongoing and results will be presented at the conference.

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P02.058.D The 16q22.1 fragile site: a novel player in female infertility?

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Background: Common fragile sites (CFS) are genomic regions prone to breakage under DNA replication stress, therefore impairments in DNA damage repair pathways may exacerbate this fragility. While CFS are known to be involved in various genetic disorders, aside from the fragile X premutation, their role in primary ovarian insufficiency (POI) is not fully understood. Chromosome band 16q22.1 contains two CFS, whose connection to infertility is uncertain. This study aims to investigate whether POI patients may exhibit higher chromosome instability (CI) due to impaired NHEJ, HR, and ATM DNA repair pathways.

Material and methods: CI was evaluated on blood samples from 7 POI patients with negative exome and *FMR1* premutation to analyze the DNA repair pathways. A control was included for each sample. Analysis was performed on 100 metaphases from each experiment. Each cell was scored for the number and type of CI abnormalities.

Results: No statistically significant differences were found in NHEJ, HR, or ATM-dependent DNA repair pathways between POI patients and healthy controls. Intriguingly, among the POI patients, the sample displaying the greatest number of breaks across all experimental conditions exhibited a fragile site at 16q22.1, as revealed by conventional cytogenetic analysis.

Conclusion: While our sample size limits definitive conclusions, the observed correlation between the fragile site and increased genomic instability deserves further investigation. It is important to determine whether these breaks in POI patients, both with and without the fragile site, consistently occur at 16q22.1. A compromised DNA repair gene in this region may contribute to POI.

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P02.059.A Detection of a novel small non-coding RNA in seminal plasma of fertile men

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Background: Small non-coding RNAs (ncRNAs) in seminal plasma are known as potential biomarkers for diagnosing and differentiating types of male infertility. This study focuses on detection of a novel ncRNA, namely novel_seq32, in seminal plasma of normal fertile men for the first time. This sequence was firstly identified by authors in the previous research in human testis tissue.

Material and methods: Twenty seminal plasma samples were collected in accordance with WHO guidelines. The total RNA was isolated using a column kit (Pars Toos, Iran), yielding concentrations between 130 and 250 ng with a 260/280 absorption ratio of 1.7 to 2. Quantitative Real-Time PCR (qRT-PCR) was utilized to validate the presence of this novel sequence in seminal plasma. U6 was selected as the reference gene, and miR-888-5p was utilized to validate qRT-PCR accuracy. Bioinformatics analysis was performed.

Results: The mature sequence of novel_seq32 is GTCACAAATTCGTATCTAGGGGA (- strand on Chr17, Start: 46657214, End: 46657303). The sequence was detected in all samples by qRT-PCR. It also was blatted to a region human genome including HOXB3 and HOXB-AS3 genes. This finding indicates a gene regulatory cluster involving in cell growth and differentiation.

Conclusion: These findings suggest that novel_seq32 has potential as a biomarker for impaired parameters in semen analysis and forensic studies in the future.

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P02.060.B A novel homozygous *PSMC3IP* variant in a patient with hypergonadotropic hypogonadism and short stature

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Background: The *PSMC3IP* gene on chromosome 17q21.2 encodes a protein crucial for meiotic recombination. Biallelic pathogenic variants in *PSMC3IP* are linked to ovarian dysgenesis (OD), a genetically heterogeneous disorder causing amenorrhea and streaked gonads. Despite advances in next-generation sequencing (NGS), the etiology of OD remains unknown in most cases.

Case report: An 11-year-7-month-old girl presented with short stature. She was born at term with a birth weight of 3000g, and her parents were first-degree cousins with no history of premature menopause or infertility in the family. Her height and weight were 137.4 cm(−1.9SDS), and 39.1 kg(−0.3SDS), respectively. Midparental height was -2.3SDS. She was prepubertal with no systemic abnormalities, and bone age was delayed (10 years). Gonadotropins were elevated with, low estradiol, and reduced AMH. Pelvic ultrasonography failed to visualize the uterus and ovaries. Hearing and cardiac assessments were normal. GH stimulation testing revealed GH deficiency (GHD). The pituitary MRI was normal. Due to poor growth velocity, rGH therapy was commenced. Puberty progressed to Tanner stage 3 spontaneously. Chromosome analysis was 46,XX. Clinical exome sequencing identified a novel homozygous *PSMC3IP* variant, NM_016556:c.261dup/p.(Val88-Serfs*12), classified as likely pathogenic and not found in gnomAD. Both parents were heterozygous.

Conclusion: We report a novel variant in *PSMC3IP* causing OD and GHD by expanding the phenotypic spectrum of this condition, suggesting short stature as a novel feature. Additional cases are needed to refine the phenotype, and wider use of NGS will improve diagnostic accuracy.

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P02.061.C Recurrent pregnancy loss and chromosomal anomalies: insights from a single-center analysis in Turkey

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Background: Recurrent pregnancy loss (RPL), defined as the loss of two or more consecutive pregnancies before the 20th to 24th week of gestation, affects approximately 1–5% of couples. Constitutive chromosomal abnormalities are a significant cause of RPL, as chromosomal imbalances in the gametes of carriers may lead to embryonic lethality. This study aimed to investigate chromosomal abnormalities in couples with a RPL history.

Material and methods: We conducted a retrospective analysis of the medical records of 678 couples(1356 patients) who were admitted to Van Education and Research Hospital between 2017 and 2024. Peripheral blood lymphocyte karyotyping was performed using GTG banding techniques following standardized protocols.

Results: Chromosomal aberrations were detected in 4.12% (28/678) of couples, aligning with the 2–5% prevalence range reported in the literature for RPL populations. Among affected individuals, 57.1% (16/28) had structural abnormalities, including 12 balanced translocations, two inversions, and two Robertsonian translocations. Numerical chromosomal abnormalities were detected in 12 individuals (42.9%, 12/28), including four cases of 47,XYY, 47,XXX, or marker chromosome abnormalities. The remaining eight cases exhibited mosaicism, with sex chromosome mosaicism observed in females and marker chromosome mosaicism in one male and one female. These findings are consistent with previously reported data in Turkey.

Conclusion: Chromosomal anomalies, particularly translocations and rare mosaicisms, play a critical role in RPL etiology. Cytogenetic analysis is essential for genetic counseling and optimizing reproductive outcomes. These findings emphasize the necessity of comprehensive genetic investigations to elucidate RPL pathogenesis and develop targeted management strategies.

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P03 Prenatal Genetics

P03.001.D Whole exome sequencing (WES) increases the molecular diagnosis of 106 foetuses with structural anomalies negative at chromosomal microarray analysis

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Background: Foetal structural abnormalities (3% of all pregnancies) range from isolated to multisystemic malformations. WES analysis has recently provided an accurate molecular diagnosis when chromosomal microarrays (CMA) failed to identify their genetic aetiology.

Material and methods: 106 foetuses presenting structural malformations and negative to CMA analysis were recruited. After multidisciplinary consultation and detailed phenotypical characterisation, in trio WES analysis on genomic DNA extracted from chorionic villi, amniotic fluid or fetal tissue obtained after pregnancy termination was performed. Minigene assay was conducted to functionally characterise selected splicing variants of uncertain significance (VUS).

Results: 51% of the foetuses in our cohort presented with multisystemic malformation, while 49% presented a single major malformation (i.e. fluid accumulation, urogenital, cardiac, skeletal and central nervous system anomalies). WES analysis, based on clinical features according to the human phenotype ontology terms, identified 37 variants in 31 genes, the majority (46%) being classified as likely pathogenetic. Two selected splicing variants in *INPPL1* and *RHOA* genes, initially classified as VUS, were functionally characterised by minigene assay thus confirming their pathogenetic effect. Notably, a novel missense variant in *ZNF292* gene was firstly detected in a prenatal setting. Altogether, the WES diagnostic yield was 34.9% (37/106 foetuses), with the highest rate for urogenital and skeletal anomalies.

Conclusion: This study supports the utility of an integrated clinical and molecular approach in the challenging Italian prenatal setting, offering couple counseling and recurrence risk estimation. Furthermore, the implementation of minigene assay in the laboratory routine could improve the characterisation of clinically relevant non-coding variants.

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P03.002.A Targeted long-read sequencing of native DNA for genetic disease diagnostic and screening research

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Background: Despite enormous advances in DNA sequencing, the causal mutations of many human diseases remain challenging to characterize with standard methods. Examples include fragile X syndrome and Friedreich's ataxia, caused by expansion of short tandem repeats, congenital adrenal hyperplasia (associated with a segmental duplication) and hemophilia A (caused by large inversions). The adoption of long-read sequencing has the potential to improve and simplify sequencing and analysis of these complex regions. In addition, the ability to detect methylation enables simultaneous generation of sequence-level and epigenetic data. We present new panel content for the PureTarget assay, which uses the CRISPR/Cas9 system to generate targeted sequencing libraries of native DNA, sequenced with long and accurate HiFi sequencing.

Material and methods: Coriell DNA samples with known mutations were prepared with the PureTarget assay and sequenced on Revio and Vega systems. Samples were analyzed with a novel pipeline which relies on the TRGT and Paraphrase genotyping tools.

Results: PureTarget allows for the evaluation of a panel of targets in multiplexed samples in a single assay and analysis workflow. In samples with known pathogenic mutations, we demonstrate the ability to define and resolve structurally complex repeat alleles (as needed for *RFC1*) and resolve structural variants in genes relevant for carrier screening such as *CYP21A2*. For *FMR1* this approach can simultaneously size repeat expansion, detect methylation, and identify AGG interruption sequences.

Conclusion: The PureTarget assay and software pipeline allows for a comprehensive characterization of repeat expansions and other challenging targets.

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S.B. Kingan: A. Employment (full or part-time); Modest; PacBio. A. Employment (full or part-time); Significant; PacBio. **J. Bruand:** A. Employment (full or part-time); Significant; PacBio. **J. Zhou:** A. Employment (full or part-time); Significant; PacBio. **T. Mokveld:** A. Employment (full or part-time); Significant; PacBio. **G. De Sena Brandine:** A. Employment (full or part-time); Significant; PacBio. **E. Dolzhenko:** A. Employment (full or part-time); Significant; PacBio. **M.A. Eberle:** A. Employment (full or part-time); Significant; PacBio. **D. Kilburn:** A. Employment (full or part-time); Significant; PacBio.

P03.003.B Maternal genetic effects on placental DNA methylation: first insights and potentially regulated pathways. (Award Candidate)

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Background: Placental epigenomics provides valuable insights on maternal-fetal interactions in relation to pregnancy outcomes, and health later in life. This study aimed to explore the relationship between maternal and fetal genomic data, and placental DNA methylation (DNAm) patterns by identifying *trans* and *transgenome* methylation quantitative trait loci (*trans*/*trangenome*-mQTLs).

Material and methods: We analyzed 337 placenta samples from the INMA project with the EPIC and GSA arrays from Illumina. We also genotyped the DNA extracted from the blood of the donor mothers, using the GSA array. mQTLs were generated using TensorQTL, with placental DNAm modelled as a function of genotype and covariates including DNAm and genotype principal components, cell type estimations and fetal sex. T*rans*-mQTLs, defined as associations between fetal genotype and placental DNAm (SNP-CpG distance >0.5 Mb); and *transgenome*-mQTLs, defined as associations between maternal genotype and placental DNAm, were identified.

Results: After Bonferroni correction ($p = 1.57 \times 10^{-14}$), we identified 98,553 *trans*-mQTLs, comprising 1,618 CpGs and 61,172 SNPs. The *transgenome*-mQTL analysis revealed 2,731 associations, including 107 CpGs and 2,682 SNPs. Interestingly, 82 mQTLs found in the *transgenome*-mQTL dataset did not appear in the *trans*-mQTL results, with six unique CpGs under exclusive maternal control. These CpGs were located on active chromatin and promoter regions of genes related to brain development, stress response and cell cycle.

Conclusion: These findings underscore the role of the maternal genetic background in the regulation of placental DNAm, with particular impact on key processes such as brain development and cell cycle regulation.

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P03.004.C Technical advances in non-invasive pre-implantation genetic screening at Zan Mitrev Clinic

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Background: Non-invasive pre-implantation genetic screening (niPGS) is an advanced reproductive medicine technique that enables embryo analysis for chromosomal abnormalities without biopsy. Using cfDNA extracted from the culture medium in which embryos develop, niPGS offers a safer, non-invasive alternative to traditional PGS methods which involve extracting cells from the embryo.

Material and methods: After intracytoplasmic sperm injection, the fertilized oocytes were cultured individually from day-1. On day-3, the embryos were transferred in a 10 µl blastocyst medium and cultured until day-5/6. Readapting the Ion ReproSeq PGS kit, originally designed for biopsied material, we analyzed 33 spent-medium samples from 33 pre-selected cultured embryos, plus 4 negative controls (cultured-medium blank and water).

Results: Over 100,000 sequencing reads were obtained for 76% of the samples, while negative controls had a median of 8,853 reads per sample. Data acquisition failed for 24% of samples. Using the ReproSeq kit, we detected 52% euploid, and 48% aneuploid and/or mosaic samples, demonstrating its effectiveness in identifying diverse chromosomal abnormalities in embryos. The median time from sample collection to result was 3 days.

Conclusion: Traditional PGS kits can be readapted for use in non-invasive settings with an acceptable failure rate. However, cross-validation studies with biopsied material, are essential to confirm the method's accuracy and reliability. To enhance the cost-effectiveness of niPGT, novel approaches such as Nanopore sequencing can eliminate the need for streamlining the large number of samples per sequencing run. NiPGS holds great potential as a valuable tool to increase the success rate of in vitro pregnancies.

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P03.005.D Placental mosaicism for autosomal trisomies and structural variants (Award Candidate)

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Background: Mosaicism, the presence of two or more cell lines originating from the same zygote, is identified in 2–4% of chorionic villus samples (CVS). Estimating fetal prognosis based on a mosaic CVS result is challenging. Mosaicism may indicate true fetal mosaicism (TFM), potentially causing fetal disease, or confined placental mosaicism (CPM), which can lead to placental dysfunction. This study aimed to provide aberration-specific risk estimates for TFM and adverse outcomes in cases of CPM.

Material and methods: We analysed all Danish singleton pregnancies with mosaicism involving an autosome (trisomies and structural variants) detected in CVS from 1983 to 2021 using data from the Danish Cytogenetic Central Registry and the Danish Fetal Medicine Database. Information on pre- and postnatal cytogenetic analyses and pregnancy outcomes was collected. Risk estimates for TFM, preterm birth (<37 weeks), and low birth weight (<2.3 percentile) were calculated.

Results: Among 948 identified cases, 779 (82%) had follow-up analyses.The overall TFM risk was 18% (95%CI: 15.3;20.9) and varied by aberration: common trisomies (13, 18, 21): 25% (17.2;33.2), rare autosomal trisomies (RAT): 10% (6.9;12.9) structural variants (SV): 28% (22.8;34.3). For CPM-pregnancies, 13% (10.3;16.5) gave birth before 37+0 (common trisomies: 10% (4.0;19.0), RAT: 16% (12.1;21.2), SV: 9% (5.0;15.1)) and 12% (8.9;14.8) had low birth weight (common trisomies: 4% (0.9;12.0), RAT: 16.6% (12.4;21.6), SV: 6% (2.5;10.9)).

Conclusion: Risk of TFM is highly dependent on the specific aberration, which should be communicated to pregnant couples along with the substantial risk of adverse outcomes in CPM for specific aberrations.

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P03.006.A How to sustain genome sequencing in prenatal diagnosis in less than 7 workdays? : the French PRENATOME-ULTRA pilot study

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Background: Prenatal management of congenital malformations (3% of pregnancies) relies on rapid causal diagnosis. Chromosomal microarray (CMA) and exome sequencing (ES) face delays of 2–6 weeks and are often used sequentially, which remains burdensome for couples. Since genome sequencing (GS) can simultaneously identify single, copy number, and structural variants, its use in prenatal diagnosis as a first-line test with suitable turnaround-time (TAT) could be of great interest. A French pilot study evaluates the feasibility and diagnostic yield of GS as a first-line test with a TAT of ≤7-workdays.

Methods: Ultra-rapid GS was performed alongside CMA/ES in 90 pregnancies (8 centers) with either two anomalies or one with strong suspicion of genetic cause. Only ACMG class 4/5 variants were considered as positive diagnosis.

Results: To date, GS TAT of ≤7-workdays was met for 49/68 (72%) (median 6.5 workdays without 2 outliers caused by variant validation to exclude false-positive, compared to a median of 10 and 15 workdays for CMA and ES, respectively). The transport and wet lab phase was the most common cause of delay, although other limitations were encountered: excessive bioinformatics step (2), report generation (2). The diagnostic rate was 17/68 (25%). In addition, 11 interesting variants of uncertain significance were reported to clinicians. GS was highly concordant with CMA/ES (98.5%).

Conclusions: These preliminary results demonstrate that prenatal GS is feasible with a TAT of ≤7-workdays and similar diagnostic yield to ES/CMA combined. However, such ultra-rapid TAT presents significant constraint. All inclusions will be completed by the time of ESHG.

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P03.007.B Redefining pre-conception screening: rare disease detection and equity through couple whole exome sequencing

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Background: The standard ethnicity-based carrier screening (EBS) in Israel has successfully reduced the prevalence of diseases, such as Tay-Sachs in Ashkenazi Jews and Infantile neuroaxonal dystrophy in Israeli Bedouin. However, this targeted approach is

Table 1. P/LP variants detected by pre-conception couple exome screening (pCCE) across 483 families from diverse ethnic groups in Israel.

	Number of pCCE-detected events	% of events missed by standard ethnic-based carrier screening	Number of pCCE-detected genes	% of Genes missed by standard ethnic-based carrier screening	Number of Couples/ Individuals with pCCE-detected Event	% of Couples /Individuals missed by standard ethnic-based carrier screening
Shared AR (OMIM)	75 (bi-allelic P/ LP variants)	73/75 (97.3%)	36	26/36 (72.2%)	67 (Couples)	65/67 (97%)
Autosomal Recessive (AR)	1,335	1,102/1,335 (82.5%) (genes missing in EBS)	386	255/386 (66.1%)	480 (Couples)	426/480 (88.7%) (genes missing in EBS)
X-linked (XL)	10	10/10 (100%)	8	8/8 (100%)	10 (Couples)	10/10 (100%)
Expanded secondary findings panel	155	NA	60	NA	147 (Individuals)	NA

Shared AR (OMIM) includes all P/LP mutations across all recessive OMIM genes. **Autosomal Recessive (AR)** includes P/LP mutations detected in either parent among the Israeli Extended Gene Panel. **X-linked (XL)** includes clinically significant XL disorders. **Expanded secondary findings panel** of 180 actionable genes, including the American College of Medical Genetics and Genomics secondary finding list (ACMG SF v3.2).

inherently limited, overlooking rare variants and genetic risks in diverse populations.

Methods: We developed a pre-conception couple exome screening (pCCE) approach screening for pathogenic (P) and likely pathogenic (LP) variants in the autosomal recessive (AR) and X-linked (XL) disorders, and additionally reporting expanded secondary findings panel with potential health implications for the couple.

Results: We retrospectively analyzed 483 consecutive couples of diverse ethnicities collected at a single center between 12/2023-01/2025 using the pCCE approach. Our results, summarized in Table 1, highlight the inclusiveness of detectable P/LP variants by pCCE compared to previously used EBS. Our pCCE detected 75 shared carrier status in AR disorders, spanning 36 different genes. Notably, EBS has no representation for 26/36 (72.2%) of these genes, and 73/75 (97.3%) of the specific variants detected. Additionally, EBS missed all XL variants 10/10 (100%) and 155 expanded secondary findings pCCE results.

Conclusion: pCCE offers three critical advantages: (1) Detecting rare diseases that limited-gene panels fail to detect; (2) Facilitating ethnicity-agnostic variant screening of genetic risks across all populations; (3) Providing couples with actionable insights about their health through the secondary findings. Taken together, our approach enables informed reproductive choices while alleviating ethnicity bias and allowing for a higher yield in capturing P/LP in diverse populations.

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P03.008.C Analysis of DNA methylation abnormalities in chorionic villi in obstetric syndromes

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Background: The physiological course of pregnancy is supported by the functioning of many cell types, among which the chorion performs functions that ensure normal placentation. Aberrant DNA methylation, shown in some obstetric syndromes, is another factor that disrupts this process. Given the pathogenetic similarity of the syndromes, it is assumed that there are common methylation patterns for these pathologies, with unique patterns observed in spontaneous abortions (SA) representing an extreme phenotype.

Material and methods: Chorionic DNA methylation profiles of spontaneous (n = 7) and induced abortions (n = 7) were analyzed using reduced representation bisulfite sequencing. Methylation data for preterm labor, preeclampsia and intrauterine growth restriction were obtained using Genome Expression Omnibus public data repository - GSE100197, GSE74738, GSE115508, GSE197795. Differentially methylated genes (DMGs) were obtained for each syndrome. Common and specific DMGs were then identified and annotated. Expression of selected genes was confirmed by immunohistochemistry.

Results: *CLEC16A* was hypermethylated for all pathologies and 237 DMGs were specific for SA. The functional annotation of these specific DMGs revealed a cluster involved in the genesis and function of primary cilia and another cluster associated with epidermal growth factor. DMGs involved in embryonic development (*CMIP, EGFR, FBN2, GLI3* and *PBRM1*) showed different expression in chorionic villi of SA compared to induced abortions.

Conclusion: The analysis revealed a substantial number of DMGs specific to SA. The identification of gene clusters may indicate a functional relationship with impaired embryonic development, and changes in gene expression in SA suggest a potential role in the formation of a lethal phenotype.

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P03.009.D Ultrasound and genetic diagnosis of fetal skeletal dysplasias in a fetal cohort

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Background: Skeletal dysplasias are a heterogeneous group of genetic disorders resulting from mutations in more than 500 genes involved in bone formation, mineralization, and cartilage development. We report the ultrasound and genetic results of 27 cases of fetal skeletal dysplasias, retrospectively collected from 2019 to 2024.

Material and methods: Selection was based on women who underwent prenatal genetic counseling at the Policlinico S. Orsola Genetics Unit for ultrasound findings revealing skeletal dysplasia. Genetic tests including karyotype, CMA analysis, targeted gene testing and NGS panels were performed.

Results: The average gestational age at the first suspicion of skeletal dysplasia was 20+6 days, though severe cases showed abnormalities such as hydrops and micromelia since the first trimester. Among the 21 women who underwent invasive procedures, 15 (71.4%) received a molecular diagnosis. Five cases had pathogenic mutations in type I and II collagen genes. Three cases involving *KIAA0753, HSPG2, DYNC2H1* genes were recessive, with heterozygous variants inherited from both parents. One fetus carried a maternally inherited X-linked pathogenic variant in the *EBP* gene, while the remaining diagnoses were de novo mutations.

Conclusion: Skeletal dysplasias are primarily genetic disorders, however their large number and phenotypic variability makes diagnosis complex. The combination of detailed ultrasound assessment and advanced genetic analyses enhances diagnostic accuracy. Early detection allows for more informed reproductive choices, and a confirmed genetic diagnosis can impact recurrence risk assessments for affected couples.

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P03.010.A How to implement whole genome sequencing in pregnancies with fetal structural anomalies

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Background: Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) are becoming more widely available in genetic disease diagnosis. However, data on their clinical utility and integration into clinical management during pregnancy remain limited.

Material and methods: We report our 9-year experience implementing prenatal WES and WGS in a retrospective cohort of 322 fetuses with ultrasound anomalies, both in ongoing pregnancy (43,5%) and terminated pregnancy (56,5%). Two hundred thirty-five trio-WES and 87 trio-WGS were performed on DNA extracted from fetal samples and parents' peripheral blood. Fetal phenotypes were classified into predefined subgroups based on the sonographic findings at the time of sample collection and/or autopsy findings.

Results: The overall diagnostic yield was 32.4%, varying between different clinical categories. We evaluated the integration and timing of WES/WGS into the prenatal diagnostic care pathway, the clinical utility, the potential impact on pregnancy management, the outcome (especially regarding continuation or interruption of pregnancy on the result), and parents' reproductive decisions.

Conclusion: Our experience supports using WES/WGS in ongoing pregnancies, confirming their high potential for identifying the molecular etiology of fetal ultrasound anomalies. However, it also shows several challenges associated with their use in a prenatal setting, including complexities associated with clinical phenotyping, technical considerations, timing, and interpretation of results. Both pre- and post-test genetic counseling in the context of a multidisciplinary team remains crucial in giving indications for analysis and in communicating the test results with implications for the fetus and the couple.

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P03.012.C The diagnostic yield and genetic findings of prenatal whole exome sequencing of fetal sonographic abnormalities: A retrospective study from Qatar

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Background: Prenatal karyotype (KT) and chromosomal microarray (CMA) are diagnostic in ~9% and ~6% of cases with fetal sonographic abnormalities, respectively. An additional 20% to 47% of cases could be diagnosed using prenatal whole exome sequencing (WES). We aim to investigate the diagnostic yield of prenatal WES in Qatar, since its implementation in 2014.

Material and methods: More than 2,150 patients registered in the prenatal genetic clinic in Qatar from Oct 2014 to Dec 2023 were screened. Cases were eligible if they underwent prenatal WES after nondiagnostic KT and/or CMA. WES findings were reviewed and classified.

Results: A confirmed genetic diagnosis was observed in 65/143 cases (45.5%). A total of 68 different pathogenic or likely pathogenic variants in 49 genes were identified, of these, 73.8% were AR. The most common condition was Nemaline Myopathy, seen in 21.5% (n = 14) of solved cases. Ten of those cases belonged to consanguineous Qatari families from the same tribe and were caused by the same homozygous pathogenic variant in the *NEB* gene, supporting a potential founder effect. The reassessment of variants of uncertain significance resulted in the re-consideration of three variants in *CLEC16A, VTA1 and ATP1A2* as being likely clinically relevant.

Conclusion: Prenatal WES has significant potential as a diagnostic tool in the prenatal setting in Qatar, with one of the highest reported diagnostic yields worldwide. Our study also recommends offering optional targeted testing for the *NEB* variant at premarital screening, considering the notable incidence of this variant in Qatar compared to elsewhere.

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P03.013.D Diagnostic and clinical utility of prenatal exome sequencing: an Italian experience

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Background: Ultrasound scans detect fetal structural abnormalities in approximately 3% of pregnancies. Exome sequencing (ES) identifies a monogenic disorder in 26–36% of cases. Few studies have explored the clinical utility of ES in the prenatal setting.

Material and methods: This retrospective study involved two enrolling centers and a single laboratory; 131 pregnancies with single or multiple fetal anomalies, with uninformative karyotype and chromosomal microarray results, were analyzed with ES at any gestational age. Cases were categorized into 13 phenotypic subgroups. 59 pregnancies were terminated and 72 resulted in live births. The primary endpoints were assessing diagnostic utility (diagnostic yield and clinical diagnosis reclassification) and clinical utility (prognosis definition, impact on pregnancy management,

postnatal care, and familial counseling), evaluated using a questionnaire completed by healthcare professionals before and after the analysis.

Results: The overall diagnostic yield was 29.8% (39/131). ES had an impact in both positive and negative cases: positive results led to diagnostic reclassification in 59% of cases (23/39) and prognosis definition in 51.3% of cases (20/39); negative results impacted diagnosis and prognosis in 18.9% (17/90) and 36.7% (33/90) of cases, respectively. Familial counseling was modified in 92.3% of positive cases (36/39) and 57.8% of negative cases (52/90). Postnatal care was modified in 85.7% of positive cases (12/14). Pregnancy management was less influenced by ES results.

Conclusion: The use of ES in the prenatal setting offers significant clinical utility, regardless of the results (positive or negative), mainly by aiding in diagnosis classification, prognosis definition, improving postnatal care, and enhancing familial counseling.

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P03.014.A Investigating mosaicism for monosomy X in the placenta and fetus: a registry-based study of cases from Denmark 1983–2021

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Background: Mosaicism, defined by a mix of chromosomally normal and abnormal cells, is detected in up to 4% of chorionic villus samples (CVS). When detected in CVS, the abnormal cells can either be generalized to the fetus (true fetal mosaicism, TFM) and potentially cause fetal disease or confined to the placenta (confined placental mosaicism, CPM) which may lead to placental dysfunction.

This study examines the potential outcomes following the detection of monosomy X mosaicism in CVS.

Material and methods: We collected data on all singleton pregnancies with mosaicism for monosomy X in CVS between 1983–2021 from two nationwide registries: Danish Cytogenetic Central Registry and Danish Fetal Medicine Database.

Results: During the study period 90,973 CVSs were registered. Of these we identified 204 cases of mosaicism for monosomy X; 111 cases were CPM and 93 cases were TFM. The overall risk of TFM was 46% (93/204, 95% CI: 39;53). In cases with CPM, the risk of SGA was 7% (6/84, 95% CI: 3;15) and the risk of preterm birth was 10% (8/82, 95% CI: 4;18). The mean level of mosaicism in the CVS was 38% (95% CI: 33;44, n = 74) in case of CPM and 55% (95% CI: 47;62, n = 52) in case of TFM.

Conclusion: Mosaicism for monosomy X detected in CVS carries a high risk of TFM. The risk of SGA and/or preterm birth in relation to CPM is, however, low. As expected, the mean level of mosaicism found in the CVS is higher in cases with TFM compared to CPM.

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P03.015.B Non-invasive prenatal testing: a retrospective analysis of confirmatory cytogenomic findings in high-risk pregnancies

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Background: Non-invasive prenatal testing (NIPT) based on the analysis of cell-free fetal DNA in maternal plasma has sensitivity rate of approximately 99%, with false positive results below 1%. However, the positive predictive values (PPVs) have been shown to be much lover, except for trisomy 21.

Material and methods: In a five-year period a total of 201 women where referred for invasive prenatal diagnosis due to high risk or uninformative results obtained after NIPT. Chorionic villus sampling was performed in 70 cases, and amniocentesis in 131 cases. Cytogenomic analysis was performed by karyotyping, fluorescence in situ hybridization and chromosomal microarray analysis (CMA).

Results: The PPVs for detection of the most common trisomies -T21, T18 and T13 were 96.5% (110/114), 76.5% (13/17) and 46.5% (6/13), respectively. For the sex chromosome aneuploidies including 45,X, 47,XXY, and 47,XXY, PPVs were 12.5%, 100% and 100%, respectively. Among 7 cases with inconclusive results for sex chromosomes, in only one case a sex discrepancy between ultrasonography and cytogenomic analysis was found. In the case of rare chromosomal aneuploidies (RCAs) PPV was 14,3% (1/7), and in the case of structural copy number variations (CNVs) was 13.3% (2/15). There were 15 uninformative NIPT results and the cytogenomic analysis revealed normal results in all cases.

Conclusion: NIPT provides high PPVs in screening for T21, thereby increasing its prenatal detection and reducing the number of invasive procedures. On the contrary, a low proportion of confirmed results for 45,X, RCAs and CNVs strongly implies confirmatory diagnostics, and cautious management of such pregnancies.

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P03.016.C Identification of a HomozygousVariant in the *CYP21A2* Gene through cell-free fetal DNA analysis via NGS

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Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder with an incidence of 1 in 10,000–15,000 live births. In 90–95% of cases, it is caused by 21-hydroxylase deficiency due to *CYP21A2* mutations, leading to increased adrenal androgens. Affected females may present with ambiguous genitalia, while males often exhibit precocious puberty. Non-invasive prenatal testing (NIPT) using cell-free fetal DNA (cffDNA) has been applied to monogenic diseases since 2012, demonstrating diagnostic potential. Our case involved a fetus classified as female and low-risk for aneuploidies via NIPT but born with ambiguous genitalia, raising suspicion of CAH.

Material and ethods: Karyotyping confirmed a 46,XX genotype, consistent with the NIPT result. We performed PCR and reverse dot-blot analysis of *CYP21A2* on DNA from the patient and both parents. Retrospective next-generation sequencing (NGS) of stored maternal plasma from the time of NIPT assessed the feasibility of detecting the mutation in cffDNA.

Results: The c.290-13A/C>G (I2 splice) mutation was identified in homozygosity in the patient and in heterozygosity in both parents. NGS of cffDNA detected the same mutation with a Variant Allele Frequency (VAF) of 67% and a fetal fraction of 5%.

Conclusion: The high VAF suggests cffDNA can identify point mutations and small insertions/deletions in monogenic disorders, providing a non-invasive alternative for prenatal diagnosis. Early identification of pathogenic variants affecting sexual development could enable timely interventions, including potential in utero therapy, improving neonatal outcomes.

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P03.017.D Clinical experience with use of cffDNA for non invasive identification of POC chromosomal abnormalities in early pregnancy loss

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Background: Around 25% of pregnancies result in miscarriage, with 50% caused by chromosomal abnormalities. Identifying these causes has prognostic value for couple's and mother's reproductive health, as euploid miscarriages are associated with maternal dysfunctions. However, obtaining products of conception (POC) is invasive, and traditional methods like karyotyping have low success rates and high costs. Genetic analysis is typically performed after multiple miscarriages, highlighting the need for non-invasive diagnostic alternatives. This study aimed to evaluate the use of circulating free fetal DNA (cffDNA) for genome-wide screening of chromosomal abnormalities in early miscarriages.

Material and methods: Plasma samples from women with POC retained or expelled within 24 hours were processed using the VeriSeq NIPT Solution v2 and sequenced on the NextSeq 550Dx. Data were analyzed with the VeriSeq NIPT Assay Software v2 and an in-house bioinformatics algorithm.

Results: A total of 366 samples were analyzed: 202 euploid and 164 aneuploid. Among the aneuploid samples, 20 had trisomy 15, 38 had trisomy 16, 21 had trisomy 22, and other rare autosomal aneuploidies (RAAs) were detected, including trisomies of chromosomes 2, 3, 4, 5, 6, 9, 10, 14, and 20.

Conclusion: Chromosomal abnormalities were found in 45% of cases, with trisomy 15, 16, 22, and monosomy X representing 28% of these. The non-invasive nature of this method offers diagnostic opportunities even after the first miscarriage, improving the diagnostic process and aiding in the development of new preventive and therapeutic strategies.

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P03.018.A Incremental yield of exome sequencing in fetuses with isolated polyhydramnios: diagnostic insights and clinical impact

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Background: Exome sequencing (ES) shows potential in identifying genetic causes of polyhydramnios, but systematic evaluation and guidelines are lacking. This study aimed to assess the diagnostic yield of ES in isolated polyhydramnios.

Material and methods: This retrospective multicenter study included 40 cases of isolated polyhydramnios (AFI ≥25 cm or SDP ≥8 cm). Mild cases were defined as AFI ≥25 and <30 or SDP ≥8 and <10 cm; moderate-severe cases as AFI ≥30 or SDP ≥10 cm. All cases were screened for gestational diabetes mellitus (GDM) and underwent chromosomal microarray analysis (CMA). ES was offered for CMA-negative cases, along with targeted tests for spinal muscular atrophy, myotonic dystrophy type 1, and Prader-Willi syndrome.

Results: Pathogenic or likely pathogenic variants were identified in seven cases (17.5%), including four cases of Bartter syndrome (variants in *KCNJ1*, *BSND*, and *MAGED2*), one case of Noonan syndrome (*RIT1*), one case of Osteopathia Striata with Cranial Sclerosis (*AMER1*), and one case of autism/intellectual disability syndromes (*AUTS2*). Two cases had variants of uncertain significance, and one case was positive for myotonic dystrophy. Among the 17 mild cases, 2 (12%) had positive ES results. Notably, two ES-positive cases also had gestational diabetes mellitus (GDM). Postnatal follow-up revealed normal development in 89% of live-born infants, with isolated instances of global developmental and speech delays.

Conclusions: ES significantly enhances diagnostic yield in isolated polyhydramnios, even in mild or late-diagnosed cases and with GDM, supporting its integration into the diagnostic workflow.

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P03.019.B iMedImage: an end-to-end foundational model for medical image recognition and its application in chromosome analysis

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Background: Karyotyping, the gold standard for detecting chromosomal abnormalities, remains critical for diagnosing genetic and hereditary diseases. While AI effectively identifies numerical abnormalities, detecting structural abnormalities remains challenging. Leveraging advances in large foundation models, which integrate multimodal medical images for robust feature extraction and accurate diagnoses, we developed iMedImage, an end-to-end model for medical image recognition. Applied to chromosomal abnormality detection, iMedImage demonstrated promising performance.

Material and methods: We constructed a comprehensive medical image dataset encompassing diverse modalities from common medical fields, including chromosome, cellular, pathological, ultrasound, X-ray, CT, and MRI images. Based on this dataset, we developed iMedImage, which incorporates the following key features: (1) a unified representation for diverse modal inputs and medical imaging tasks, and (2) multi-level (case-level, image-level, and patch-level) image recognition capabilities, enhanced by chain of thought (CoT) embedding and mixture of experts (MoE) strategies.

Results: A prospective and retrospective, multicenter, stratified block-randomized, crossover clinical trial with a "gold standard" control, enrolling 1,518 participants, was conducted at three Class III A hospitals in China. The model achieved fully automated chromosome analysis, including segmentation, arrangement, and abnormality detection, with a diagnostic accuracy rate of 99.59%, sensitivity of 95.83%, and specificity of 100%.

Conclusion: We present iMedImage, an innovative end-to-end foundation model for medical image recognition and demonstrates its capability to significantly enhance performance in chromosomal abnormality detection tasks. Our proposed iMedImage can provide clinicians with an effective tool for identifying chromosomal abnormalities and potentially contribute to reducing birth defects.

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P03.020.C Cell-based NIPT: one test for reliable detection of the most common prenatal syndromes, including Down syndrome, 22q11 deletion syndrome, and cystic fibrosis

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Background: Currently, the most common prenatal syndromes—Down syndrome, 22q11 deletion/duplication syndrome, and cystic fibrosis (CF) —are typically assessed separately, often requiring multiple - and potentially invasive tests - per pregnancy. The isolation of extravillous trophoblasts from maternal blood provides a pure fetal DNA source, free from maternal DNA, enabling comprehensive non-invasive prenatal genetic analysis. However, this study comprises multiple smaller cohorts focusing on different aspects of testing.

Material and methods: Using a newly ISO 15189-accredited methodology, trophoblasts were isolated from maternal blood of pregnancies (GA 10–15 weeks) via magnetic-activated and fluorescence-activated cell sorting. Genetic analysis (ACGH, WGS (4x), Sanger seq) following whole-genome amplification enabled:- Aneuploidy detection- Pathogenic CNVs: >2 Mb for deletions and >5 Mb for duplications genome-wide- Smaller CNVs (>0.4 Mb) associated with known microdeletion/duplication syndromes- Targeted analysis of the 50 most common CF-causing CFTR variants- A bespoke setup for monogenic disorders

Results: Validated in a public-private collaboration in Denmark 500+ pregnancies have been analyzed across different study arms:- cbNIPT screening for 50 common CFTR variants demonstrated 27/28 (96%) concordance with invasive testing, one without trophoblasts.- Chromosome 22q11.2 CNVs (0.4–2.8 Mb) was the most common CNV and was identified in eight pregnancies, highlighting genomic instability from low-copy repeats (LCRs A-H) and the phenotypic importance hereof.- 15 monogenic disorders (20 cases) were successfully tested in pregnancy

Conclusion: cbNIPT is ready for clinical use, providing a non-invasive alternative for aneuploidy and CNV detection. Soon follows a CFTR screening and bespoke setup, reducing the need for multiple tests per pregnancy.

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P03.021.D The clinical significance of prenatal whole genome sequencing

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Background: This study investigated the diagnostic yield of short-read whole genome sequencing (WGS) in prenatally detected congenital malformations and the consequences of the results.

Material and methods: A prospective study of 100 fetuses with various malformations, negative for trisomies and copy number variants, which were analyzed with fetal parental trio WGS.

Results: A molecular diagnosis was achieved in 26/100 (26%). The phenotypes were divided into different groups with the following diagnostic yields: multisystem 10/25 (40%), CNS 9/25 (36%), skeletal 4/13 (31%), thorax 2/20 (10%), abdominal 1/11 (9%) and other isolated anomalies 0/6 (0%). Of the cases with a causative variant, 18 (69%) were terminated due to the prenatal ultrasound findings before the results from WGS were available, one was terminated after the pathological WGS result, four ended in perinatal death and three resulted in a live-birth. 19/26 (73%) of the causative variants were de novo. However, 7/26 (27%) of the causative variants had a significant recurrence risk with 3/26 (12%) variants being autosomal recessive, 3/26 (12%) autosomal dominant with a carrier parent and 1/26 (4%) X-linked. 5/7 (71%) of the couples with a significant recurrence risk chose PGT for their next pregnancy.

Conclusion: We demonstrate an additional diagnostic yield of 26% with WGS in prenatally detected congenital malformations where chromosome aberrations were previously excluded. 27% of the detected variants had a significant recurrence risk. Hence, WGS increases the diagnostic yield in current pregnancies, but also increase the number of couples with a known significant recurrence risk in future pregnancies.

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P03.023.B Increasing clinical utility of prenatal exome sequencing: the potential power of Artificial Intelligence phenotype-driven analysis

Maayke A. de Koning[1], *Mariëtte J.V. Hoffer*[1], *Ilse Feenstra*[2], *Malou Heijligers*[3], *Helger Ijntema*[2], *Galuh Astuti*[2], *Mariet Elting*[4], *Mariëlle E. Van Gijn*[4], *Quinten Waisfisz*[4], *Nicole Corsten-Janssen*[5], *Birgit Sikkema-Raddatz*[5], *Andrea C. Postmus*[5], *Klaske D. Lichtenbelt*[6], *Ellen Van Binsbergen*[6], *Hanneke W.M. Van Deutekom*[6], *Gijs W.E. Santen*[1], *Manon Suerink*[1]

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Genomic diagnostics are increasingly requested in pregnancies with congenital anomalies. Identifying the causal variant while minimizing Variants of Uncertain Significance (VUS) and Unsolicited Findings (UF) is challenging since variant interpretation is complicated by limited fetal phenotypes. Artificial intelligence (AI) variant interpretation software might aid prioritizing variants.

Prenatal Exome Sequencing cases were prospectively included between February 2022 and October 2024 in a Dutch nationwide study. Exome data with HPO-terms were reanalysed using AION by Nostos Genomics. The validity of the AI platform was evaluated and rates of VUS and UF were compared with the DDG2P gene panel.

Overall, 133 samples from 131 pregnancies (two dizygotic twins) were reanalyzed. During routine care, variants related to the prenatal phenotype were reported in 24 cases (18%) of which $n = 3$ VUS and UF in 5 cases (4%) of which $n = 1$ VUS. The mean number of allocated HPO-terms per case was 2.5 (SD 1.7), with AI prioritizing 11.0 variants (SD 5.2) per case. Linear regression showed no significance between the number of HPO-terms and prioritized variants ($p = 0.792$). AI ranked the diagnostic variant in the top 5 in 96.6%. The total number of VUS did not significantly differ between usage of the DDG2P panel and AI ($p = 0.250$), however significantly more UF were detected with AI ($p = 0.039$).

AI is a promising add-on in the often-laborious interpretation of variants and has the potential to make curating gene panels redundant. This is crucial in prenatal care, where the increasing number of referrals and time pressure of advancing gestation are challenging.

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P03.024.C Exome sequencing in structurally normal fetuses - yield, lesson learned and complexities

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Background: Exome sequencing (ES) is a highly effective and well-established method for detecting disease-causing single nucleotide variations (SNVs) and small indels in postnatal cases. In prenatal situations, where the clinical characteristics of the affected fetus can often be vague and non-specific, ES plays a crucial role in diagnosis. However, for structurally normal fetuses, the benefits are less well-defined. There is limited information available on the utility and complexities of exome sequencing in this population.

Material and methods: From February 2017 to June 2024, ES was performed in 1608 structurally normal fetuses. Analysis included SNV, copy number variations and uniparental disomy. Only pathogenic/likely pathogenic variants, based on ACMG classification criteria, were reported.

Results: ES was carried out between 16–22 weeks of pregnancy. 12 of the 1608 fetuses (0.75%) were found to have pathogenic variants in genes related to moderate to severe diseases. Autosomal recessive diseases were diagnosed in three fetuses (25%). Autosomal dominant diseases were diagnosed in eight fetuses (67%), all of which de-novo. An X-linked inherited disease was diagnosed in one fetus (8%). Most of the diagnosed diseases have a prominent neurodevelopmental phenotype, hence not detected by ultrasound. There were another two groups of results, related to a phenotype with an actionable importance related to fetal and child health (five fetuses) or a mild phenotype (four fetuses).

Conclusion: With restrictive analysis and careful management of the results, prenatal ES might be beneficial when offered in adjunct to genetic counseling in structurally normal fetuses. Neurodevelopmental phenotypes were enriched in this cohort.

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P03.026.A Investigation of *SIK3* and *CEP164* variant pathogenicity in human fetuses with severe skeletal defects

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Background: This research is part of the Genomic Autopsy study, an Australian initiative investigating the genetic cause of perinatal deaths. Two homozygous VUS were identified in *SIK3* (c.1178G>A, p.Arg393His) and *CEP164* (c.393+6T>G) in two human fetuses presenting with polydactyly, shortened long bones and severe ossification irregularities. SIK3 regulates long bone growth. Disruption of SIK3 kinase activity causes skeletal abnormalities. Mutations within *CEP164* give rise to ciliopathies, which have broad clinical phenotypes, including skeletal. The aim of this study was to investigate the VUS using mouse genetics and NGS, to provide evidence for variant causation or benign re-classification.

Material and ethods: Homozygous *SIK3* mutant mice were assessed by whole-mount skeletal staining, histology and micro-CT. RNA sequencing of parental PBMCs was performed to assess *CEP164* transcript splicing. The *CEP164* variant was introduced in human and murine cells using Prime Editing (PE), and *CEP164* splicing assessed by RT- PCR.

Results: No differences in gross skeletal morphology or bone micro-architecture were observed in pre- and post-natal *SIK3* mutant mice relative to controls. RNA-seq revealed 50% non-canonical *CEP164* splicing in the parents, with exon 5 skipping introducing a premature stop codon. *CEP164* PE in HEK293T cells revealed transcript splicing consistent with the parental RNA-seq data.

Conclusion: These findings suggest the *SIK3* variant is likely benign, and the *CEP164* intronic mutation is likely pathogenic and causative of the skeletal deformities in the fetuses. This important work contributes to variant classification in rare genetic diseases to inform prenatal screening, genetic counselling, and ultimately reduce perinatal deaths globally.

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P03.027.B Prenatal variants of uncertain significance (VUS): to report or not to report?

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Background: Prenatal Exome Sequencing (pES) has a significant diagnostic yield but time pressure and limited phenotypic information make interpretation of Variants of Uncertain Significance (VUS) more challenging than in a postnatal setting. We share our experiences of prenatal reporting of highly suspicious VUS.

Material and methods: We retrospectively analyzed pregnancies in which VUS identified by pES were reported to parents during pregnancy in two Dutch academic medical hospitals.

Results: During the study period, 31 VUS in 29 genes were reported in 27 pregnancies. Cases were assigned to one of five groups based on consistency of prenatal phenotypes with gene-associated diagnoses. The implications of VUS included clinical evaluation of parental carriers ($n = 4$), additional screening of proband ($n = 2$), influencing parental decision-making ($n = 11$) and/or prompting confirmatory testing ($n = 10$). Reanalysis with currently available data resulted in reclassification of seven variants, five of which were upgraded to (likely) pathogenic.

Conclusion: Although we do not recommend routine disclosure, our data suggest that prenatal reporting of VUS can be valuable in exceptional cases. Stringent selection was applied and only a minority of reported VUS was reclassified as (likely) pathogenic. Therefore, a careful individual assessment of each VUS case remains imperative and multidisciplinary meetings should be an integral part of prenatal VUS management.

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P03.028.C Prenatal trio exome sequencing for fetuses with isolated nuchal translucency thickness above the 95th percentile but below 3 mm

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Background: Increased nuchal translucency (NT) thickness is associated with a higher risk of chromosomal abnormalities, as well as monogenic developmental disorders, which can be detected during pregnancy via exome sequencing. Currently, recommendations for referral to prenatal exome sequencing vary between countries, such as requiring the additional presence of fetal structural malformations, or an isolated NT above the 95th or 99th percentile, or above an absolute value of 3 or 3.5 mm. Our aim was to evaluate the diagnostic yield of exome sequencing in fetuses with NT above the 95th percentile, but below 3 mm, both with and without further structural anomalies.

Material and methods: Trio exome sequencing was performed on fetal DNA and blood collected from both parents. There were five cases of increased NT < 3 mm with further structural anomalies, and 56 cases of isolated NT < 3 mm.

Results: In 1 out of 5 of the NT cases with further anomalies, a (likely) pathogenic variant for a monogenic developmental disorder was determined. In comparison, in the isolated NT group, 11% of cases had (likely) pathogenic variants in genes causing monogenic developmental disorders, with an additional 4% showing "hot" variants of uncertain significance (VUS) and 12% showing regular VUS.

Conclusion: Given the high incidence of (likely) pathogenic variants for monogenic developmental disorders found in fetuses with isolated increased NT thickness, our findings support the use of exome screening for NT cases above the 95th percentile,

independent of the absolute measure values, and even in the absence of further structural anomalies.

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P03.029.D The utility of non invasive prenatal testing in early diagnosis of tetrasomy 12p

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Background: Non-invasive prenatal testing (NIPT) for detection of rare chromosomal imbalances has been extensively used in past years. Pallister-Killian syndrome (PKS) is a rare disorder due to tissue-limited mosaicism for isochromosome of the short arm of chromosome 12: i(12)(p10). PKS prenatal diagnosis is challenging as manifestations are variable and nonspecific (i.e. increased nuchal translucency, polyhydramnios, etc.). The utility of NIPT in the diagnosis of PKS was never explored.

Material and methods: We reviewed a subset of a previously published cohort (Faieta 2024) with NIPT positive results for 12p duplication using the CE-IVD certified Veriseq NIPT Solution V2 (Illumina, Inc. San Diego, CA, USA), together with an in-house developed algorithm. All patients were offered follow-up: karyotype on amniotic fluid (AF) and maternal lymphocytes.

Results: NIPT revealed dup(12)(p13.33q11) in 9 patients, definite follow-up was available for 6: fetal karyotype on AF confirmed the presence of isochromosome 12p [47,XX,iso(12)(p10->pter)] in 2 cases, the remaining 4 showing a normal fetal karyotype (≥50 metaphases analyzed). In one case a supernumerary marker chromosome was identified on maternal lymphocytes (mos47,XX,+mar[28]/46,XX[72]). Notably, among cases without available follow-up one declined amniocentesis and gave birth to a baby referred for neurodevelopmental abnormalities (post-natal investigation were refused). None showed ultrasound abnormalities.

Conclusion: This study demonstrates NIPT can detected 12p duplication related to PKS in early pregnancy without ultrasound abnormalities. The main limitation of the study is represented by the small number of cases, and extension of the cohort is needed to reliably assess the detection rate of NIPT for 12p tetrasomy.

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P03.030.A Secondary findings from prenatal exome-sequencing: prevalence, types of conditions and variants

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Background: Clinical use of exome sequencing for prenatal diagnosis is on the rise. Exome analysis may (or may not) include a list of genes, recommended by the American College of Medical Genetics (ACMG), predominantly associated with adult-onset conditions for which prevention/treatment/early detection exists (currently 81 genes). Guidance regarding these secondary-findings and disclosure in a prenatal setting is lacking.

Material and methods: Our in-house database of exome-sequencing data was analyzed for the prevalence of pathogenic variants in the ACMG secondary-findings genes, the type of variants identified, and related conditions.

Results: In 2023–2024, 2235 fetal exomes were performed at our tertiary center. Seventeen pathogenic variants in the ACMG secondary-findings genes were reported in prenatal testing (0.8%). Nine variants (53%) were in genes associated with cardiovascular phenotypes (APOB, LDLR, MYBPC3, RYR2, PKP2, PCSK9), and eight variants (47%) were in genes related to cancer phenotypes (BRCA1, BRCA2, MAX, TMEM127). Seven variants (41%) were founder mutations (mainly the Ashkenazi-Jewish BRCA1/2 mutations), six variants (35%) were previously reported as disease-causing, and four loss-of-function variants (24%) were not previously reported.

Conclusion: Although there are challenges in implementing secondary-findings disclosure in the prenatal setting, such findings can have positive clinical impact on parents and on their family members. Chances of identifying pathogenic variants are greater in populations with founder mutations. Pre-test counseling regarding prenatal exome-sequencing should include discussion of the inclusion or exclusion of secondary findings.

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P03.032.C Detection of copy number variations (CNVs) on chromosome 18 by non-invasive prenatal testing (NIPT): case reports of partial deletions

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Background: NIPT is a screening test used to detect trisomy of chromosomes 21, 18, and 13 through the analysis of cell-free DNA. Recently, increasing interest has been in detecting CNVs based on NIPT results. In this context, we report two cases of CNVs on chromosome 18 identified through NIPT.

Material and methods: NIPT, chromosomal microarray analysis (CMA), and karyotyping were performed.

Results: Case 1: A 33-year-old woman (G2P0) at 12+3 weeks' gestation was subjected to NIPT due to an increased nuchal translucency and a short nasal bone of her fetus. The NIPT results indicated partial deletions in 18p11.32p11.21 (15.4 Mb) and 18q21.2q23 (25.8 Mb). At 14 weeks' gestation, intrauterine fetal death was diagnosed, and the CMA results performed on the fetal tissue were consistent with the NIPT findings.

Case 2: A 33-year-old woman (G2P0) underwent NIPT at 12+1 weeks' gestation. In NIPT, partial deletions in 18q21.2q23 (25.8 Mb) were detected, and CMA and karyotyping were performed on both the fetus and the parents. The results were consistent with the NIPT findings. Additionally, CMA of the fetus revealed duplications in 8p23.2 (2.2 Mb) and deletions in 12p11.22p11.21 (2.6 Mb), while karyotyping revealed 9p12q13 inversions. Comparison with the parents' results confirmed that the deletions in chromosomes 18 and 12 were de novo pathogenic variants. Following genetic counseling, the pregnant woman chose to terminate the pregnancy.

Conclusion: NIPT may be used as a screening tool for CNVs. However, confirmatory tests, such as CMA or karyotyping, are still necessary to ensure accurate genetic counseling.

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P03.035.B Positive NIPT for trisomy 16 in an euploid fetus - the unmasking of maternal UPD 16

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Background: The widespread clinical use of noninvasive prenatal testing (NIPT) has led to increase in unexpected findings. Trisomy 16 occurs in approximately 1–2% of all clinically recorded pregnancies and is a common finding in spontaneous abortions. While full trisomy 16 is typically lethal, mosaic trisomy 16 can be viable. Postzygotic trisomic rescue may result in uniparental disomy (UPD), with diverse possible clinical outcomes. Maternal UPD 16 is associated with a spectrum of phenotypic variability, with intrauterine growth retardation (IUGR) being the most common manifestation.

Material and methods: A young couple's first pregnancy was investigated following a positive NIPT result for trisomy 16 and sonographic fetal anomalies. Microsatellite (QF-PCR) and Chromoseq analysis of amniotic fluid were performed, along with trio microsatellite analysis for UPD 16. Post-termination QF-PCR analysis was conducted on placental and fetal samples.

Results: NIPT detected trisomy 16 and male fetal sex. Genetic amniocentesis by QF-PCR demonstrated a normal male fetal chromosomal profile, while ultrasound revealed IUGR in a female fetus. Subsequent Chromoseq analysis showed a normal chromosomal result and male sex. Trio microsatellite analysis revealed absence of paternal alleles at three informative chromosome 16 markers, confirming the diagnosis of upd(16)mat. Post-termination analysis demonstrated full to low-grade mosaic trisomy 16 in the placenta, with normal fetal result. In addition to IUGR ambiguous genitalia and finger anomalies were observed.

Conclusion: This case highlights the challenges in interpreting rare trisomies detected via NIPT and underscores the critical role of interdisciplinary collaboration between clinicians and geneticists for accurate diagnosis and pregnancy management.

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P03.036.C Prenatal diagnosis of Smith-Lemli-Opitz syndrome : the critical role of multidisciplinary collaboration in a rare case

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Background: Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive disorder caused by pathogenic variants in the DHCR7 gene, leading to defects in cholesterol biosynthesis. The syndrome is characterized by facial dysmorphism, microcephaly, intellectual disability, limb abnormalities (polydactyly, syndactyly, bone shortening), multiorgan malformations. Prenatal diagnosis is challenging due to its variable phenotype.

Material and methods: An 18-week pregnant woman was referred for genetic counseling due to ultrasound findings of postaxial polydactyly in one hand and both feet, along with shortening of all long bones in the fetus. Amniocentesis was performed at 19 weeks of gestation, and whole-genome sequencing (WGS) was initiated, followed by targeted analysis of 841 genes associated with skeletal dysplasias. Additionally, genome-wide screening for variants with an expected loss of function (pLoF) was conducted. Segregation analysis was performed for the identified variants in both parents. Free estriol levels were measured at 22 weeks of gestation.

Results: WGS revealed a known pathogenic variant (c.452G>A) and a likely pathogenic variant (c.1364A>G) in DHCR7, both in heterozygous states, suggesting a diagnosis of SLOS. Segregation analysis confirmed that the c.452G>A variant was paternally inherited and c.1364A>G maternally. Maternal serum estriol levels were significantly reduced, as expected in SLOS pregnancies. Following genetic counseling, the couple opted for pregnancy termination.

Conclusion: Prenatal diagnosis of SLOS is rare due to its heterogeneous presentation and limited awareness of its early ultrasound features. This case underscores the importance of collaboration between experienced fetal morphologists and geneticists in recognizing and confirming such cases, enabling informed decision-making for affected families.

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P03.037.D Diagnostic strategy for unsolved miscarriages - results from a pilot study

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Background: About 50% of miscarriages are caused by several risk factors, such as embryo aneuploidy, anatomical abnormalities, immune response or genetic dispositions. The other 50% remain unexplained. This study uses exome sequencing (ES) to explore genetic causes, particularly in families with recurrent miscarriages, as we presume to find variants that are lethal in embryonic state and have not been found in living individuals so far.

Material and methods: We retrospectively analyzed miscarriages that previously had an unremarkable karyotype, no growth in cell culture or no clear cytogenetic evaluation. Female samples with unremarkable karyotypes were tested for maternal contamination. A total of 80 unresolved cases underwent ES, with strict filtering of single nucleotid variants and HPO terms.

Results: Here, we highlight one family with three miscarriages might linked to a maternally transmitted hemizygous variant NM_002049.4:c.919C>T, p.(Arg307Cys) in *GATA1*. This gene plays an important role in erythroid development by regulating the switch from fetal to adult hemoglobin. Several OMIM morbid conditions caused by *GATA1* variants are known. The variant segregated with all male miscarriages, leading us to classify it as likely pathogenic. Further evaluation of the healthy male child in the family is ongoing.

Conclusion: In summary, we recommend the immediate integration of ES into the diagnosis of unresolved abortions in form of trio-ES to address the limitations shown by our retrospective pilot study (1) copy-number variant analysis is not feasible in old (stored) material, (2) inheritance of new variants is not traceable and (3) phenotypic data are missing in older cases.

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P03.038.A An incidental finding in prenatal exome sequencing: a case report and ethical implications of a novel variant in Wolcott Rallison Syndrome

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Background: Targeted molecular tests in prenatal diagnosis enable early detection in families with a history of genetic disease. Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder caused by *EIF2AK3* mutations, with a prevalence of <1 in 1,000,000. This study evaluates clinical and ethical considerations when incidental findings lack phenotypic evidence in utero.

Material and methods: A 42-year-old pregnant woman and her consanguineous husband sought genetic counseling counseling due to a previous deceased child from congenital lactic acidosis at 14 months. In 2015, an inherited disease panel found no causative variant. During the current pregnancy (7 weeks' gestation), clinical exome sequencing (CES) was performed on the parents, their healthy child, and preserved DNA from their deceased child. No ultrasound abnormalities were detected. Genomic DNA from peripheral blood and fetal DNA from CVS underwent Next-Generation Sequencing (NGS). Quantitative PCR (qPCR) confirmed copy number variations (CNVs).

Results: CNV analysis identified a heterozygous *G6PC1* deletion in both parents and also homozygous in deceased child, associated with Glycogen Storage Disease Type IXc. Additionally, a novel heterozygous 38.4 kbp deletion of *EIF2AK3* was incidentally identified in both parents. CES on the fetal sample revealed homozygous *EIF2AK3* deletion, confirming WRS. qPCR performed to validate findings. Following genetic counseling, the couple decided for termination at 24 weeks.

Conclusion: This case highlights the utility of prenatal exome sequencing and CNV analysis. The incidental diagnosis of WRS underscores its clinical value, while the ethical complexities of incidental results emphasize the need for comprehensive genetic counseling in reproductive decision-making.

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P03.040.C Exploring socio-demographic factors in a 2-year cohort of patients offered rapid prenatal exome sequencing across the North East of England and Yorkshire (NEY)

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Background: In England, the national prenatal exome sequencing (pES) service offers rapid genetic testing for pregnancies with significant fetal anomalies with a likely monogenic cause. The NEY region serves a diverse population, including some of the most socio-economically deprived areas in England, rural communities, and urban areas, such as Bradford with a large ethnic minority population where consanguineous marriage is common[1]. We examined all pES cases between 01/08/2021 and 31/12/2023 to identify patterns in the use of and the impact of this specialist testing.

Methods: Retrospective analysis of patient records was undertaken in the 3 Clinical Genomics services: Leeds, Sheffield, and Newcastle. Patients were identified through genomics laboratories and clinical databases.

Results: 109 pES were completed, with 29 pathogenic or likely pathogenic variants reported. Overall 12.8% of couples were consanguineous. Diagnostic yield was higher within the consanguineous group than the non-consanguineous group (35.7% vs 26.1%). Geographical analysis showed samples received from rural and urban areas. Lower than expected test rates were observed in some areas corresponding to those with higher consanguineous populations and rates of congenital anomalies[1]. Socio-economic deprivation was not associated with low testing rates.

Conclusion: Testing rates were lower, but the diagnostic yield was higher in areas with high levels of consanguinity. Further work is required to understand if testing is not being offered or accepted, and to ensure equity of access to pES across NEY. We will explore the potential barriers and enablers of access to pES

testing that may help understand different patterns across the region.

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P03.041.D Prenatal characterization of a pathogenic variant in enoyl Coenzyme A hydratase short chain 1 (ECHS1): a case report

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Background: Pathogenic variation in ECHS1 gene is described as causative for autosomal recessive mitochondrial short-chain enoyl-CoA hydratase 1 deficiency (ECHS1D, OMIM#616277). Disturbed metabolism of branched-chain amino acids leads to lactic acidosis and is known to cause neurodegenerative Leigh-like syndrome in neonates and infants. Data on prenatal features are scarce, although abnormal corpus callosum, facial dysmorphic features and cardiomyopathy were described before. In this case of ECHS1D we describe severe neurodevelopmental delay prenatally determined by ultrasonography and MRI.

Material and methods: Case report highlighting the diagnostic pathway in a singleton pregnancy from mid-trimester ultrasound scan to prenatal diagnosis of ECHS1D.

Results: Patient was referred at 19 weeks of gestation with single umbilical artery for a detailed mid-trimester ultrasound scan. Additionally a thin, though intact, corpus callosum and interhemispheric cystic lesion were visualized. Routine follow-up ultrasound scan at 28 weeks of gestation revealed 8 weeks of delay in gyration pattern, which was confirmed by MRI; pachygyria, polymicrogyria and cysts in basal ganglia were noted, next to hepatomegaly. MRI was repeated with only minimal progression of gyration pattern. Amniocentesis was performed. Whole Exome Sequencing (WES) with trio-analysis led to the identification of a homozygous pathogenic variant in ECHS1 gene c.817A>G p.(Lys273Glu) in the fetus, causative for ECHS1D. Both parents were found to be heterozygous carriers. Pregnancy was terminated at 32 weeks of gestation.

Conclusion: Prenatal abnormal gyration pattern, cysts in basal ganglia and fetal hepatomegaly were the main features in this case leading to the prenatal diagnosis of mitochondrial short-chain enoyl-CoA hydratase 1 deficiency.

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P03.042.A Prenatal diagnosis and neonatal phenotype of a newborn with a de novo heterozygous LZTR1 gene variant

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Background: Pathogenic variants in LZTR1 are associated with schwannomatosis, a hereditary tumor predisposition syndrome, and Noonan syndrome (NS), characterized by facial anomalies, cardiac defects, developmental and growth delay, following autosomal dominant or recessive inheritance. This study reports neonatal clinical features of a NS patient with a de novo heterozygous variant in LZTR1 gene identified during pregnancy following ultrasound detection of fetal hydrops.

Material and methods: Genetic tests were performed on amniotic fluid.

Results: Fetal ultrasound at the 28th week revealed severe fetal hydrops and bilateral pleural effusion. Karyotype and array-CGH analysis were normal. NGS-based trio testing identified a de novo heterozygous missense variant, c.742G>A p.(Gly248Arg), in the LZTR1 gene, classified as pathogenic according to the ACMG criteria, causing NS. At birth, the baby presented chylothorax, large patent ductus arteriosus, renal failure, left vocal cord paralysis, severe obstructive apnea, hypothyroidism, redundant skin on the neck, hypertelorism and dysmorphic ears.

Conclusion: Fetal hydrops was the prenatal clinical sign driving specific genetic testing during pregnancy. The present case is supporting the evidence that also autosomal dominant NS caused by LZTR1 may manifest with fetal hydrops, previously reported only in the autosomal recessive form. Prenatal detection of thoracic effusion and cystic hygroma has been reported previously in two fetuses with the c.742G>A LZTR1 variant. The present report is highlighting clinical difficulties in identifying the classic NS phenotype in newborns with LZTR1 variant when cardiac defect is not present and fetal hydrops is masking characteristic facial features.

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P03.043.B Insights in underlying pathophysiology of brain malformations associated with *VRK1*-related syndrome derived from fetal neuropathology

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Biallelic variants in *VRK1* have been described in patients with heterogeneous phenotypes with childhood or adult-onset of progressive upper and lower motor neuron disease. Prenatal onset and neurodevelopmental disorders associated with *VRK1* have rarely been described in the literature. Here, we report on the first fetal cases and first neuropathological examination of the brain in patients with pathogenic variants in *VRK1*. Two fetuses from consecutive pregnancies of second-degree consanguineous parents presented with microcephaly prenatally during the early second trimester. Termination of pregnancy was performed for

both cases. Post-mortem examination showed overlapping features including facial dysmorphisms, microencephaly, agenesis of the corpus callosum, absent gyration and reduced neuronal density of the cortex, suggesting a simplified gyral pattern. One of the two fetuses showed an unusual macroscopic appearance of muscle hypertrophy that could underlie a neuromuscular disease, in the absence of histological anomalies. No visceral malformations were observed. Whole exome sequencing identified a homozygous likely pathogenic variant in *VRK1* (NM_003384.3, GRCh38):c.238C>G p.(Leu80Val) in both fetuses. The variant is located in a previously reported cluster close to the ATP-binding site. The parents were heterozygous carriers of the variant. Neuropathological examination in these cases provides first insights in the underlying pathophysiological process of biallelic pathogenic variants in *VRK1* in humans. Our findings are evocative of a combination of impaired neuronal proliferation, of a neuronal migration deficit, and abnormal axon guidance.

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P03.044.C Diagnosing mosaic variants by trio exome sequencing in termination of pregnancy fetuses

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Background: Genetic analysis in fetuses can pose different diagnostic challenges in variant discovery and interpretation. We present mosaic variants detected by trio exome sequencing (tES) in fetuses with major anomalies.

Material and methods: The cohort included three fetuses following termination of pregnancy due to major anomalies. The first fetus (male) was presented with abnormal embryonic development, abnormality of the nose, abnormal hip bone morphology, and abnormal morphology of female internal genitalia. The second fetus (female) presented with sonographic non-visualized fetal bladder and cleft lip and palate. The third fetus (male) presented with abnormal brain morphology and mild malformation of cortical development.

Results: tES analysis identified two CNVs and one SNV de novo mosaic variants:The first fetus carried a de novo mosaic duplication (70%) of chromosome 12p, suggesting Pallister Kilian syndrome. The second fetus carried a de novo likely pathogenic mosaic variant (21%) in the MED12 gene (c.2238C>A; p.Cys746*), consistent with X-linked autosomal dominant Hardikar syndrome. The third fetus carried a de novo mosaic duplication (50%) of chromosome 21, diagnostic of Down syndrome.

Conclusion: These data suggest that tES may be considered as a first-tier test for prenatal diagnosis of major fetal anomalies, detecting both CNV and SNV variants, even in mosaic form. This is of particular importance in the time-frame constraints of an ongoing pregnancy.

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P03.045.D De novo *FBN2* variant in exon 33 associated with complex congenital heart disease and hydrops fetalis: expanding the prenatal phenotype

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Background: The *FBN2* gene encodes fibrillin-2, a key structural component of connective tissue expressed in the heart. *FBN2* pathogenic variants cause congenital contractural arachnodactyly (CCA), which is characterized also by the presence of cardiac disease (primarily atrial and ventricular septal defects, aortic root dilation and valvulopathy) with no prenatal phenotype reported so far.

Material and methods: We present a case of a complex congenital heart disease detected in a fetus at 20 weeks of gestation. Ultrasonography identified cardiomegaly, right ventricular dysfunction, hypoplastic pulmonary valve with regurgitation, tricuspid valve insufficiency, abnormal ductus venosus, reversed ductus arteriosus flow, severe intra-uterine growth restriction, asymmetric cerebral ventriculomegaly and hydrops fetalis. Maternal infectious disease screening showed positive IgM and IgG against Parvovirus B19. Genetic analyses were performed on DNA from abortive tissue and from the couple's blood samples: Array-CGH ruled out genomic imbalances in a female fetus; targeted exome sequencing identified a *FBN2* de novo variant: p.(Thr1428Ile), absent in GnomAD and previously unreported in the scientific literature.

Results: Recent reports suggest that *FBN2* variants in exons 31–35 may be associated with an increased risk of structural heart disease, along with CCA. The variant we found is located in exon 33 and for this reason is strongly suspected to be associated with fetal anomalies and classified as likely pathogenic.

Conclusion: This report provides further evidence of genotype-phenotype correlation for *FBN2* and suggests a possible role of exons 31–35 pathogenic and likely pathogenic variants in the fetal presentation of structural heart disease.

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P03.046.A *ALX4*-related enlarged parietal foramina: a challenge in prenatal diagnosis

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Background: Enlarged parietal foramina (EPF) (OMIM *609597) are rare, symmetric, oval defects of parietal bones located on each side of sagittal suture. Although EPF are usually asymptomatic, some patients show symptoms such as headache, vomiting, pain over the exposed cerebral cortex and seizures. Prenatally diagnosed EPF are typically incidental findings, with encephalocele or myelocele being the most common differential diagnoses. We report a prenatal case with pathological ultrasound findings suggestive for occipital meningocele. Fetal MRI showed a rounded appearance of the cranial profile in the bilateral parieto-occipital area with possible meningeal herniation and without evident herniation of brain tissue.

Material and methods: Genetic tests were performed on amniotic fluid.

Results: Karyotype analysis and array-CGH analysis were normal. NGS-based analysis identified a de novo heterozygous c.653G>A (p.Arg218Gln) variant in the ALX4 gene. At birth, the infant was noted to have fluctuating masses at the bilateral parietal region. Brain MRI revealed the "presence of two areas of osseous discontinuity at biparietal region, associated with meningoencephalocele".

Conclusion: Case reports on EPF are crucial for advancing our understanding of this condition, especially since prenatal diagnosis remains exceedingly rare. Dominant heterozygous mutations in the ALX4 (OMIM *605420) gene is associated with EPF with high penetrance and phenotipic variability. EPF is often a benign disorder; knowledge on this condition can prevent performance of unnecessary tests and treatments, reducing the anxiety of parents. Furthermore, this work underlines the value of NGS analysis as an essential component in prenatal diagnosis, in medical management and parental decision making.

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P03.048.C Is a large deletion always pathogenic? 8p23.2 deletion in a healthy family

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Background: Chromosomal 8p23.2-pter microdeletions are extremely rare and to date only twelve patients have been reported. Clinical manifestations include mild-to-moderate cognitive impairment, dysmorphism, and neurobehavioral issues[1]. Recently, an individual with 8p23.2-pter microdeletion and normal development was reported[2]. We present a family with three asymptomatic individuals with 8p23.2-pter microdeletions identified as part of follow up testing after a prenatal diagnosis.

Objectives: To expand the clinical knowledge of individuals with 8p23.2-pter microdeletions to provide better genetic counseling to families with this finding.

Results: Amniocentesis was performed on a healthy 32-year-old female at 20 weeks gestation due to low b-HCG detected in a triple screen test (0.11 MOM). Chromosomal microarray (CMA) showed a female fetus with 6,025 KB deletion at 8p23.3-8pter.

The microdeletion was inherited from the fetus's healthy, normally developed mother, who had only mild toe malformations. Further testing done via fluorescent in situ hybridization (FISH) revealed that the microdeletion was also present in two of the fetus's siblings, both healthy children with normal development, ages 2 and 10 years. Additional testing established the microdeletion as de-novo in the mother. The couple opted to continue with the pregnancy. At age 4 months, the baby girl is a beautiful baby, with normal development.

Conclusion: We present a healthy female and her three children with normal development all carrying an 8p23.2-ter microdeletion. Together with the current information available in the literature regarding individuals with this large deletion, this report may help genetic counseling in future cases.

C.O.I. Disclosure:

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P03.049.D Identification of a new phenotype combining anencephaly and fronto-nasal dysplasia associated with *ZIC2* pathogenic variants

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Neural tube defects (NTDs) are congenital malformations that arise during the third week of embryogenesis, characterized by the incomplete closure of the neural tube, the precursor of central nervous system (CNS). Anencephaly represents the cephalic form of NTDs. The transcription factor *ZIC2* plays a crucial role in CNS patterning during development and is known contributor to holoprosencephaly, a congenital brain malformation caused by the incomplete division of the forebrain into two hemispheres during early development. Here, we report the first two cases of anencephaly associated with fronto-nasal dysplasia (FND) related to pathogenic variant in *ZIC2*.

The first patient was diagnosed with anencephaly by ultrasound during pregnancy, and autopsy additionally revealed FND. The pregnancy was a monozygote twin gestation. The second fetus was diagnosed with semi-lobar holoprosencephaly after birth. The second case also involved anencephaly and FND. In the first family, SANGER sequencing identified a de novo heterozygous frameshift pathogenic variant in *ZIC2*, p.(Cys398Argfs*18) which was also present in the twin. In the 2nd case, exome sequencing identified a heterozygous de novo missense variant in *ZIC2*, p.(Asn349Ser), with pathogenic predictions from in silico *analysis*.

We report here for the first time a novel phenotype associated with ZIC2 by describing two cases of anencephaly associated with FND. Our findings suggest that mutations in ZIC2 can lead to diverse phenotypic outcomes, ranging from holoprosencephaly to abnormal neural tube closure with FND. Consistent with this, Zic2 mutant mice exhibit, in addition to the holoprosencephaly, delayed neurulation, which results in neural tube closure defects.

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P03.050.A Rare prenatal finding : *AKT1* somatic variant for which phenotype ?

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Background: Prenatal reports of pathogenic *AKT1* (OMIM*164730) variants are extremely rare, with limited descriptions of prenatal manifestations. The most frequently reported features include limb malformations, grey matter heterotopia, brain parenchymal anomalies, and megalencephaly.

Material and methods: A woman was referred to our center at 23 weeks of gestation (WG) for macrocrania exceeding the 99th percentile. A finding confirmed at 23 WG+5, along with a pronounced parieto-occipital sulcus. At 31 WG+5, fetal MRI revealed megalencephaly, temporal polymicrogyria, and atypical operculization of the Sylvian fissures. The pregnancy was continued.

Results: The array performed on native amniotic fluid (AF) was normal. Trio exome sequencing on native AF revealed a de novo heterozygous mosaic missense variant of unknown significance in *AKT1* (NM_001382430.1:c.235C>A, p.(Gln79Lys)) with a variant allele frequency of 26%. Deep NGS sequencing confirmed post-zygotic transmission. Cultured AF cells revealed a selective advantage, allowing the variant to be reclassified as likely pathogenic.

Conclusion: We report this *AKT1* somatic variant at a prenatal stage for the first time. Previously, it was described as pathogenic in a child with AKT1-related overgrowth syndrome without any reported cerebral manifestations, and differs from the recurrent *AKT1* variant (c.49G>A, p.(Glu17Lys)) in Proteus syndrome. While the clinical and molecular correlation remains uncertain, pathogenicity cannot be excluded. Could the variability of AKT1-related disorders indicate a broader spectrum of overgrowth syndromes ? Given the identification of a mosaic *AKT1* variant, we highlight the importance of analyzing both native AF and cultured AF cells for accurate variant classification.

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P03.052.C Genomic and epigenomic analysis of fetal hydrocephalus in ARID1A-associated BAFopathy: potential role of aberrant non-sense mediated RNA decay

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Germline variants in *ARID1A* have been associated with the so-called BAFopathies, including Coffin-Siris syndrome, which is characterized by microcephaly, hirsutism, and small nails and is associated with a unique episignature. Hydrocephalus has not been considered part of BAFopathy until recently, when *ARID1A* variants were implicated in prenatal-onset hydrocephalus. It remains unknown whether *ARID1A*-associated hydrocephalus is linked to a specific class of variants and whether it exhibits an episignature comparable to that of BAFopathies. We conducted genomic and epigenomic analyses on a fetus diagnosed with severe hydrocephalus on prenatal ultrasound at 19 weeks. After detailed counseling, the pregnancy was terminated at 21 weeks. The delivered fetus exhibited hirsutism and small nails. With approval from the ethics committee and appropriate consent, exome sequencing using umbilical cord blood identified a de novo heterozygous frameshift variant in the last exon of ARID1A (NM_006015.6:c.5259_5262dupGTCT, p.(Ser1755Valfs*2)). In parallel, episignature analysis was conducted using nanopore sequencing and a support vector machine-based classifier. The epigenetic profile was consistent with BAFopathy. The frameshift variant in the last exon is expected to escape nonsense-mediated decay (NMD). Although NMD has not been previously linked to ARID1A-related hydrocephalus, a retrospective review of published cases identified at least three fetuses with hydrocephalus who likely had disrupted NMD due to truncating ARID1A variants in the last or penultimate exons. In conclusion, ARID1A-associated hydrocephalus occurs within the broader clinical and epigenomic spectrum of BAFopathy but involves a distinct genetic mechanism, including aberrant NMD surveillance, which had not previously been recognized as a potential contributing factor.

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P03.053.D Socioeconomic Status and Termination of Pregnancy for Trisomy 21

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Background: Socioeconomic status (SES) influences the use of the health-care system. The potential association between SES and the propensity for choosing to terminate a pregnancy following a prenatal diagnosis of trisomy 21 is, however, not well-documented. Knowledge on such associations may help us provide unbiased information to all patients regardless of SES.

Material and methods: Registry-based study including all pregnant women with a trisomy 21 diagnosis based on prenatal invasive test from 1995–2018. The study population was defined from the Danish Fetal Cytogenetic Register. Exposure was defined as SES one year prior to the invasive test. Outcome was either termination or continuation of pregnancy, extracted from The Danish Fetal Medicine Database, The National Patient Register, The Medical Birth Registry and The Danish Cytogenetic Central Register.

Results: Of 2 591 women, 4.3% chose to continue their pregnancy after a trisomy 21 diagnosis based on an invasive test. Socioeconomic status was associated with the choice of continuing or terminating pregnancy, e.g, OR of continuing pregnancy was 4.45 [2.26 ; 8.74] for women or families with low occupational classification compared to women or families with high occupational status. The association was modified by age, ethnicity and parity of the women, but an association remained in adjusted analyses.

Conclusion: SES was associated with the propensity of choosing to continue a pregnancy with a trisomy 21 diagnosis. The result is of clinical and public health significance even though

the direction of the association, may be limited to health care systems similar to Denmark.

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P03.054.A Characterization of two cases of pyruvate dehydrogenase E1-alpha deficiency with prenatal presentation

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Background: Pyruvate dehydrogenase E1-alpha deficiency (PDHAD) is a X-linked neurometabolic disorder caused mostly by variants in PDHA1 gene encoding the E1-alpha subunit. The clinical spectrum is broad, ranging from fatal neonatal lactic acidosis to chronic neurologic dysfunction with structural brain malformations. There is a high proportion of heterozygous females with severe phenotypes. Only a few prenatal case reports have been published.

Material and methods: We describe two cases of PDHAD presenting prenatally. In the first case, both monozygotic female fetuses of a dichorionic twin pregnancy (with prior family history and prenatal diagnosis of classic simple virilizing adrenal hyperplasia) presented at 21 weeks with bilateral cavitations of the ganglionic eminence, partial agenesis of the corpus callosum (cc) and cerebellar hypoplasia. The second case was a single female fetus presenting with growth restriction at 23 weeks, who at 27 weeks showed cc atrophy and extensive periventricular destructive lesions with brain volume reduction, compatible with neuronal migration anomalies, confirmed by MRI. Both couples decided for TOP.

Results: Quad (first case) and trio (second case) WES were performed, revealing, respectively, a de novo heterozygous likely pathogenic missense variant, and a de novo heterozygous pathogenic frameshift variant in PDHA1 gene, leading to a diagnosis of PDHAD in both cases. Fetal autopsies corroborated the prenatal findings.

Conclusion: The brain abnormalities detected in our cases have been previously described. These cases highlight the relevance of performing WES in fetuses with CNS abnormalities, and allow further characterization of the rare prenatal presentation of PDHAD. This may help establish genotype-phenotype correlations.

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P03.055.B Prenatal diagnosis of MSL2-related ventriculomegaly in association with an inherited 15q13 microduplication

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The Male-Specific Lethal 2 Homolog (MSL2) gene was recently reported to be responsible of a novel neurodevelopmental syndrome, with two reports describing 28 patients all showing some form of developmental delay, around half of whom with brain MRI abnormalities, including ventriculomegaly. Reported mutations were mostly nonsense or frameshift, arguing for haploinsufficiency and loss-of-function intolerance. We hereby report the first prenatal diagnosis of a MSL2-related pathology, notably a male fetus with a priori isolated bilateral moderate ventriculomegaly, harboring a de novo heterozygous splice mutation in *MSL2*, the first splice mutation to be reported for this gene: c.142+1G>T, r.142_143ins[u;142+2_142+88], p.(Gly48-Valfs*53). The mutation was characterized through an RNA study on amniotic fluid cells and shown to lead to a non-functional truncated protein, consistent with loss-of-function intolerance. The patient also harbored a paternally inherited 1.97-Mb recurrent microduplication at 15q13.2-q13.3, known to be of incomplete penetrance and variable expressivity, but not associated with ventriculomegaly. Postnatally, the proband presented with persistent triventricular dilatation, a narrowed aspect of the aqueduct of Sylvius, square-shaped lateral ventricle frontal horns, but favorable 6-month clinical outcome. Clinical and radiological follow up will be crucial to better define the impact of the *MSL2* mutation and 15q13 microduplication on his development. This case illustrates the challenges in predicting postnatal outcomes for genetic anomalies linked to recently characterized phenotypes with limited documented cases. Characterization of more patients with *MSL2* mutations will contribute to better understanding of penetrance and phenotypic diversity. Genetic counselling in this case is further complicated by a second distinct genetic anomaly.

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P03.056.C Demographics influence on Down syndrome incidence and prenatal screening in Saint-Petersburg

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Background: The total first trimester Down's syndrome screening (FTS) in Saint-Petersburg was performed since 2012. An original bioinformatics algorithm for NIPT is used in contingent screening for women with DS risk from 1/101 to 1/1000 since 2021. **Aim**. To study the influence of migration, maternal age and screening strategy on the frequency of Down syndrome outcomes and to characterize problems that should be taken into account in city prenatal service.

Material and methods: The results of a survey of 2,083 women in the case of pre- and postnatal trisomy 21 detection in St. Petersburg from 2013 to 2023 were analyzed. Statistical processing was carried out using standard software.

Results: In 2013 the share of mothers aged 25–29 was 38.6% and decreased to 24.7% in 2023, and the group of 35–39 years were doubled (from 12.8 to 24.3%). In 11 years, the proportion of mothers aged 40 and over has been raised in 3 times. An efficiency of the city prenatal service has been increased from 60% to 94%. There is a trend in increase of gestational age at invasive

testing. 16% of women among prenatally diagnosed cases and 9% - among Down syndrome newborns did not undergo the FTS. The average incidence of Down syndrome cases (detected pre- and postnatally) increased from 1/412 to 1/258 newborns.

Conclusion: It is necessary to transfer invasive diagnostics to earlier pregnancy. An increase in the maternal age and migration processes affected the frequency of Down syndrome in Petersburg.

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T.K. Kascheeva: None. **E.S. Shabanova:** None.

P04 Sensory disorders (Eye, Ear, Pain)

P04.001.D Insights into the role of long noncoding RNA in inherited retinal disorders

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Background: Inherited retinal diseases (IRDs) are genetic disorders characterized by retinal degeneration and progressive visual impairment. The main feature of these disorders is the high clinical and genetic heterogeneity that represent a limitation for the application of gene specific therapies. For this reason, mutation-independent therapeutic approaches are gaining interest. In this direction, we focused our attention on noncoding RNAs, and in particular on long noncoding RNAs (lncRNAs). In recent years there has been an increasing amount of data suggesting their role in ocular diseases. For this reason, we aimed at generating a lncRNA expression atlas in the retina in different mouse models of inherited retinal diseases using Next Generation Sequencing-based approaches.

Material and methods: We performed single cell isolation from the retinas of rd10, RHO-P347S and WT mice using Fluorescence Activated Cell Sorting. Next we carried out RNAseq analysis on the main stages of retinal degeneration mainly focusing on rods.

Results: We identified a dataset of lncRNAs displaying the highest expression levels at the analyzed stages and identified some of them being differentially expressed between wild type and degenerating retina.

Conclusion: Different expression levels of lncRNAs between healthy and diseased retina at different stages of disease may indicate their involvement in pathological processes in IRDs. Some of the identified lncRNAs or their coding partners have been already reported in retinal development and degeneration (e.g., Rncr4, Fgf2os, Prox1os), highlighting the need for further studies on their precise role in IRDs.

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P04.002.A Loss of the Golgi stacking protein GRASP65 (GORASP1) causes glycosylation and mitotic defects associated with deafness, muscle cramps, vitreoretinopathy and white matter abnormalities (Award Candidate)

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Background: GRASP65 is a Golgi-associated peripheral protein encoded by the GORASP1 gene and is required for Golgi ribbon integrity in mammals. In vitro and in vivo models suggest that the protein also plays a key role in glycosylation and in regulating the entry of cells into mitosis, but the gene has never been associated with any human pathology.

Material and methods: Trio WGS identified a human pathogenic variant in the GORASP1 gene in a patient with a neurodevelopmental disorder combining white matter abnormalities, progressive deafness, myalgia episodes with muscle cramps but without rhabdomyolysis, severe myopia with vitreoretinopathy, and skeletal abnormalities. Cognitive assessments revealed poor verbal performance with severe dyslexia, and attention deficit hyperactivity disorder. Patient fibroblasts as well as RPE cells in which a similar mutation was introduced by CRISPR/Cas9 were used for functional studies.

Results: The variant is homozygous and causes a frameshift resulting in a stable transcript but a total absence of the GRASP65 protein. The structure of the Golgi apparatus did not show fragmentation, but glycosylation anomalies such as hyposialylation of membrane glycoproteins were detected in patient cells. Mitosis analyses revealed an excess of prometaphases and metaphases with polar chromosomes, suggesting a delay in cell cycle. These cellular defects were recapitulated in mutant RPE cells.

Conclusion: Together, these findings indicate that loss of GRASP65 in humans causes a novel Golgipathy associated with defects in glycosylation and mitotic progression. The identification of new patients will provide a better understanding of the pathology and the GRASP65 functions involved.

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P04.003.B Clinical applications of long-read sequencing: enhancing diagnosis and guiding decision-making in inherited retinal disorders (Award Candidate)

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Background: Inherited retinal disorders (IRD) are a heterogeneous group of rare diseases that cause progressive and irreversible vision loss. Despite advances in next-generation sequencing (NGS), 30–40% of IRD cases remain without a definitive genetic diagnosis, limiting clinical decision-making. This study aims to

implement a long-read sequencing (LRS) approach to improve diagnostic yields in selected IRD cases previously studied by short-read sequencing with negative or inconclusive results. In addition, the challenging ORF15 region of *RPGR* was also examined in X-linked cases.

Material and methods: Different LRS approaches using MinION or PromethION devices were applied to study 45 individuals from 27 IRD families, including amplicon sequencing (22 families), adaptive sampling (3 families), and whole-genome sequencing (2 families). Tertiary data analyses were conducted using an in-house bioinformatics pipeline. Candidate variants detected were validated through PCR and Sanger sequencing.

Results: This approach enabled a new genetic diagnosis in three previously unsolved families by identifying challenging structural variants (SVs), including retrotransposon insertions and duplications. Additionally, LRS confirmed biallelism in 11 families, suggesting an autosomal recessive inheritance, and enhanced detection while facilitating family segregation studies of *RPGR*-ORF15 variants in 11 families. One straightforward clinical application of these findings has been enabling access to Luxturna gene therapy for a patient with *RPE65* variants.

Conclusion: Our results highlight the potential of LRS to improve genetic diagnostics, facilitate targeted treatments, and support informed clinical and reproductive decision-making for IRD patients and their families.

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P04.004.C Mobile element insertions in vision disorders case descriptions in a diagnostic setting

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Background: Genome sequencing is becoming part of routine diagnostics, providing a powerful tool for comprehensive variant calling. Many complex and intronic genomic variations have been overlooked previously, including mobile elements insertions (MEI). MEIs have been shown to play a role in disease mechanisms however, they have been identified in only a small fraction of patients.

Material and methods: Here, we describe the identification of MEIs in multiple cases with vision disorders, as detected by our diagnostic pipeline and integrated tool Mobile Element Locator Tool (MELT). Variants were confirmed using long-range amplicon sequencing.

Results: In 2024, our diagnostic laboratory implemented genome sequencing as the standard technique for analyzing gene panel for vision disorder. We have performed approximately 750 analyses of differential vision disorder. In these analyses, we identified multiple cases where MEIs explained the clinical symptoms of patients. Among these, we found a heterozygous Alu insertions in exon 8 of ZEB1, resulting in autosomal dominant posterior polymorphous corneal dystrophy (OMIM #609141). Additionally, two cases from the same family with heterozygous Alu insertions in exon 45 of USH2A, which, together with different heterozygous SNVs, cause Usher syndrome (OMIM #276901). Lastly, a homozygous retrotransposon LINE-1 insertion in exon 8 of IQCB1, resulting in Senior-Loken syndrome (OMIM #609254).

Conclusion: Our case descriptions demonstrate that genome sequencing, which allows for complex variation detection, such as MEIs, enhances the diagnostic yield for patients with vision disorders. In this cohort we detected a causal MEI in 0.5% of analyses, which is higher than currently reported in literature

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P04.005.D Lysosomal macular dystrophy is a novel ocular phenotype associated with biallelic variants affecting AP-5/SPG11/SPG15 complex

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Background: Inherited retinal diseases (IRDs) are amongst the most common Mendelian human disorders in humans. Their clinical and molecular diversity is increasingly being unveiled, with more than 346 disease genes and loci identified to date. This study

aimed to characterize the phenotypic spectrum of a cohort of patients with IRD associated with mutations in genes encoding different subunits of the vesicular Fifth Adaptor Protein (AP-5) complex, a newly-identified cause of IRD.

Material and methods: Retrospective analysis of clinical data focused on ophthalmic findings from a cohort of patients with IRD attributed to biallelic mutations in *AP5Z1*, *AP5M1*, or *AP5B1*, including retinography, optical coherence tomography, and fundus autofluorescence imaging.

Results: A total of 22 patients representing 19 families were analyzed. Initial symptoms were mostly related with central vision. Younger individuals presented with asymptomatic or paucisymptomatic macular deposits, while older patients demonstrated variable areas of outer retinal and retinal pigment epithelium atrophy, with significant visual acuity loss. Retinal imaging revealed progressive enlargement of the atrophic area in cases with available follow-up data. Additionally, 10 patients exhibited concomitant neurological findings.

Conclusion: Ocular findings in our cohort illustrate a phenotypic continuum consistent with both the maculopathy described in Kjellin syndrome and sporadically reported *AP5Z1*-associated retinal findings. SPG11, SPG15, and AP-5 complex are known to interact in order to maintain endolysosomal homeostasis. We therefore propose to designate this novel IRD phenotype, resulting from molecular defects within this functional complex, Lysosomal Macular Dystrophy.

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P04.006.A Efficacy of FACS-based dual fluorescence reporter assay in developing antisense oligonucleotide therapy of novel *PRPF3* intronic splice variant

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Background: The development of antisense oligonucleotide (AON) therapy for monogenic diseases such as retinitis pigmentosa (RP) is effective but labor-intensive. There is an urgent need for rapid, efficient technologies to select optimal AONs. We herein introduce a novel methodology to efficiently test and select therapeutic AONs, focusing on intronic splicing mutations.

Material and methods: We identified a novel heterozygous intronic non-canonical splice variant in *PRPF3* causing dominant RP in a four-generation pedigree. Using a Dual Fluorescence Reporter Assay, we transfected HEK273T cells with a reporter plasmid containing either the wildtype intron (resulting in GFP fluorescence due to normal splicing) or the mutant intron (showing no green fluorescence due to splicing defects). As an alternative to standard AONs, we used uridine-rich 7 (U7) small nuclear RNA (snRNA) derivatives with optimized Sm protein-binding site and a modified antisense sequence (U7 Sm OPT snRNA) as modulators of pre-mRNA splicing.

Results: Eight AONs were designed to target the aberrant *PRPF3* transcript, to restore normal splicing and gene function. To evaluate treatment efficacy, HEK273T cells with the reporter GFP plasmid were co-transfected with U7snRNA cassettes, each containing an incorporated AON sequence. FACS analysis quantified splicing modulation and gene expression rescue, identifying the most effective AON.

Conclusion: Our findings demonstrate the potential of the FACS-based Dual Fluorescence Reporter Assay in selecting AONs for treating splice site mutations, exemplified by a novel *PRPF3* mutation. This study broadens genetic understanding and highlights the significance of personalized medicine in treating genetic disorders.

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P04.007.B 400 families' whole genome sequencing in early onset hearing loss: the French Reference Center's experience

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Background: Up to recent years, diagnosis of the genetic aetiology of early onset hearing loss(EOHL) relied on targeted gene panel analyses, with a diagnosis rate of about 50%. French clinical geneticists have now access to trio Whole Genome Sequencing (WGS) through the Plan France Medecine Genomique 2025 sequencing platforms for most patients presenting with EOHL.

Material and methods: Testing criteria for WGS differ according to the subgroup of EOHL indications. The strategy is genome-first for syndromic hearing loss (HL), WGS after normal array-CGH for HL associated with malformation(s) and WGS after normal GJB2/GBJ6 and HL gene panel for patients presenting with isolated HL. From 2020 to mid-2024, 400 families assessed in the French Reference Center forgenetic HL underwent whole genome sequencing on the SeqOIA platform.

Results: The diagnosis yield was 40%. We will present the main genes identified, the diagnostic rate according to subgroups of the EOHL indication, clinical vignettes, along with the advantages and limitations of our strategy.

Conclusion: WGS allows for identification of additional causes (class 4 and 5 variants) and candidate genes, but variants interpretation is more complex than exome or gene panel analysis. We show with our four-and-a-half years' experience that WGS is an enticing new tool for patient diagnosis, with its advantages and setbacks.

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P04.008.C *Nr2f1* mutant mouse models of Bosch-Boonstra-Schaaf optic atrophy syndrome reveal retinal ganglion cell dysfunction and optic nerve alterations

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Background: Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) is a rare autosomal dominant neurodevelopmental disorder, caused by heterozygous pathogenic variants in the transcription factor *NR2F1*, resulting, among others, in optic nerve hypoplasia and visual impairments. To explore the underlying mechanisms of BBSOAS, we generated a mouse model carrying a patient-specific missense variant (*Nr2f1*$^{+/R139L}$). Here, we performed a comprehensive characterisation of the visual system to evaluate the phenotypic impact of this mutation, alongside the previously established *Nr2f1*$^{+/-}$ mouse strain.

Material and methods: A novel *Nr2f1*$^{+/R139L}$ mouse model, harbouring a critical patient-specific missense variant in the *Nr2f1* DNA-binding domain, was introduced. Visual function was assessed in this strain in comparison to *Nr2f1*$^{+/-}$ and wild-type mice. Full-field flash and flicker electroretinograms (ffERGs) under photopic and scotopic conditions were performed to evaluate photoreceptor, bipolar cell and amacrine cell function. Pattern ERGs (pERGs) were additionally performed to ascertain retinal ganglion cell (RGC) functionality. Furthermore, retinas and optic nerves were histologically analysed to correlate functional impairments with structural changes.

Results: Mice carrying a variant exhibit significant alterations in pERG recordings compared to wild-type littermates, indicative of RGC dysfunction, while ffERGs reveal intact photoreceptor and bipolar cell function. This is supported by decreased survival of RGCs in *Nr2f1*$^{+/R139L}$ retinas, as well as compromised integrity of the optic nerves.

Conclusion: Our findings highlight selective disruptions in RGC survival and function in *Nr2f1* mutant mice, recapitulating key features of BBSOAS. These results provide new insights into the structural and functional pathology of BBSOAS, offering a platform for testing novel therapies.

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P04.009.D *STRC* findings in 670 Danish probands with hearing impairment and improved genetic testing using long-read sequencing

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Background: Hearing impairment (HI) is genetically heterogeneous with >140 genes for non-syndromic HI. Pathogenic variants in *STRC* encompassing the DFNB16 locus is now considered the most common cause of autosomal recessive mild-to-moderate HI across populations. However, interpreting *STRC* sequence data based on short-read NGS methodologies is challenging due to existence of the pseudogene, *STRCP1*, with 98% identity with *STRC*. Furthermore, a majority of *STRC*-associated HI is associated with CNV events (partial, whole, and multi-gene deletions).

Methods: From 2017–2025, 670 index cases with HI and variable additional clinical features underwent custom-made SureSelect gene panel analysis or Whole Genome Sequencing (WGS) in clinical settings. The samples were analyzed using virtual gene-panels of 35–196 genes or 101–506 genes, for patients suspected of having non-syndromic and syndromic HI, respectively. Long-range PCR, MLPA, and long-read sequencing using Oxford Nanopore Technologies were used to further evaluate SNVs and deletions.

Results: In this cohort the prevalence of *STRC*-associated hearing loss was 7% (48/670). Thirty-four patients (71%) had homozygous *STRC* deletions, also involving the entire *STRC-CATSPER2* region on one or both alleles, four patients (8%) were homozygous or partial homozygous for deletion of *STRC* only, and ten patients (21%) had a deletion in combination with a *STRC* SNV, six are novel. Nanopore sequencing was used to confirm that variants mapped with certainty to *STRC* and not *STRCP1*.

Conclusion: The results show a major contribution of DFNB16 to non-syndromic HI in the Danish population. Selected cases/families are presented. Further studies to characterize the *STRC* deletion breakpoints are in progress.

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P04.010.A Functional characterization of splicing variants in syndromic and non-syndromic hereditary hearing loss using minigene splicing assays

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Background: Hereditary hearing loss (HHL) is a genetically heterogeneous disorder, with over 150 genes implicated in non-syndromic forms and more than 400 syndromes linked to syndromic cases. Even if whole-exome sequencing (WES) has significantly improved the diagnostic rate, many cases remain unsolved due to missing heritability, partly from overlooked splicing variants. Integrating WES with functional assays can enhance variant classification, improve diagnostic accuracy and reveal hidden splicing defects in HHL.

Material and methods: This study used the SpliceAI tool to assess the impact on splicing of intronic variants identified by WES in HHL patients. Then, in vitro validation through minigene splicing assays has been performed.

Results: Eight novel intronic variants in *OTOF*, *ADGRV1*, *ATP11A*, *GSDME*, *OTOGL*, *PPP1R12A* and *USH2A* genes were identified in 8 HHL patients by WES. Minigene assays confirmed SpliceAI predictions, showing that the variants disrupted normal splicing inducing exon skipping or the selection of cryptic splice sites (ss). Notably, two *OTOF* (NM_001287489.2) variants were particularly significant: the c.5533+13G>T variant activated a cryptic 5′ss, causing partial intron retention, while the c.5104-3C>G variant led to complete exon skipping. Both variants resulted in a shift of the open reading frame and the generation of a premature termination codon.

Conclusion: This study identifies and functionally validates eight splicing variants in HHL genes, all of which disrupt RNA

splicing. These findings enhance our understanding of HHL's molecular basis and are particularly relevant to the *OTOF* gene research, given recent advancements in gene therapy.

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P04.011.B What was once unseen is now crystal clear: *CHAF1A* is a novel gene for OAVS

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Background: Oculoauriculovertebral spectrum (OAVS) is a developmental disorder primarily characterized by hemifacial microsomia and asymmetric ear anomalies, including microtia and preauricular skin tags. Its pathogenesis is complex, involving both environmental and genetic factors. In recent years, *MYT1* and *SF3B2* have been identified as monogenic causes of OAVS, while *CHAF1A*, a gene involved in chromatin assembly, has only recently been proposed as a novel contributor to this spectrum. Here, we present the second family in the literature with multiple affected individuals, consisting of a father and his three daughters, demonstrating autosomal dominant inheritance of a novel *CHAF1A* mutation.

Results: All four affected individuals presented with OAVS features, including hemifacial microsomia, microtia, and hearing loss, with the father and the youngest daughter exhibiting the most severe phenotypes. Notably, this daughter displayed striking middle ear abnormalities, such as agenesis of the malleus, incus dysplasia, and absence of the stapedial crura, along with malocclusion, and required multiple ear reconstruction surgeries. Whole-exome sequencing in 2018 failed to reveal a causative variant. However, periodic reanalysis in 2024, incorporating updated literature, identified a novel heterozygous *CHAF1A*(NM_005483.3) frameshift mutation (c.922_931dup;p.Pro311GlnfsTer16) in the father, subsequently confirmed in all affected daughters by Sanger sequencing.

Conclusion: This study not only concretely establishes *CHAF1A* as a novel gene responsible for OAVS but also expands its phenotypic spectrum to include middle ear abnormalities, a feature not previously associated with this gene. Furthermore, our findings underscore the transformative power of periodic reanalysis, even after extended periods, in uncovering hidden genetic etiologies in unresolved cases.

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P04.012.C Retinal dystrophy caused by biallelic variants in *PDSS2*, a gene involved in the coenzyme Q10 biosynthesis

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Background: Inherited retinal dystrophies (IRDs) are a group of rare diseases characterized by the progressive degeneration of the photoreceptors and/or the retinal pigment epithelium. Biallelic variants in genes involved in the biosynthesis of the coenzyme Q10 (coQ10) have been associated with coQ10 deficiency, leading to different clinical features, including syndromic and non-syndromic IRD in some patients. This work focuses on the *PDSS2* gene, which is part of this biosynthesis pathway, and whose association with retinitis pigmentosa remains largely unexplored.

Material and methods: Patients were studied by next-generation sequencing, and clinical data were obtained from self-reported ophthalmic history recorded from questionnaires, ophthalmic examinations and their clinical reports. ChimeraX was used to visualize the 3D PDSS2 structure predicted by AlphaFold for novel missense variants.

Results: We have identified six *PDSS2* biallelic patients, who had been diagnosed with retinitis pigmentosa with/without hearing loss, ataxia and/or nephrotic syndrome. This study reports eight *PDSS2* variants (four of them novel), including five missense, two splicing and one stop-gain variants. The identified missense variants are located at the end of certain alpha-helixes of the protein, and are predicted to destabilize the protein structure. Additionally, most missense variants are located in a nearby region in the predicted 3D structure of the PDSS2 protein. Interestingly, the novel variant NM_020381.4:c.365C>G is recurrent in this cohort.

Conclusion: These findings could improve clinical management of patients and allow families to receive genetic counselling. Additionally, this work supports the inclusion of *PDSS2* in screening gene panels for patients with retinal disorders.

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P04.013.D Implementation of a polygenic risk score in diagnostic genetic testing for age-related macular degeneration

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Background: Age-related macular degeneration (AMD) is a major cause of vision loss in older adults. Genetic factors greatly contribute to AMD risk. Polygenic risk score (PRS) combines multiple frequent genetic variants to predict AMD risk and development.

Methods: Baits for the intronic variants of the AMD-PRS, as described by Fritsche et al. (2016, PubMed:26691988), were included in the Twist Exome 2.0 plus Comprehensive Exome Spike-in Kit (TWIST Bioscience). Exome sequencing was conducted on an Illumina NovaSeq 6000. Zygosity and quality of all 52 PRS variants were assessed using an in-house developed tool. Variants were genotyped as homozygous wild-type (VAF < 20%), heterozygous (VAF = 20–80%), or homozygous alternative (VAF>80%). The risk score for each variant was calculated by multiplying the zygosity (0, 1, or 2) by its associated risk. If quality criteria were not met (total reads <10 or alternative reads <5), risk was estimated using allele frequency. The final PRS was the sum of all variant risks.

Results: The AMD-PRS test was evaluated in 1000 random samples. This cohort had an average PRS of 0.42 and the PRS follows a normal distribution. The allele frequencies of the 52 variants are similar to those in the European population of GnomAD v4.1.0. In 0.5% of samples, PRS could not be reliably determined due to <95% of variants passing quality criteria.

Conclusion: The developed genetic test can reliably determine the AMD-PRS in a diagnostic setting. The PRS has been incorporated into our gene panel analysis for AMD to provide a comprehensive genetic test for AMD.

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P04.014.A Overcoming challenges in the sequencing of *RPGR* ORF15 in X-Linked Retinitis Pigmentosa

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Background: Retinitis pigmentosa (RP) represents the prevalent form of retinal dystrophy, with X-linked RP (XLRP) accounting for 10–20% of cases. XLRP are characterized by progressive vision loss in males and wide range of severity in female carriers. The *RPGR* gene is implicated in over 70% of XLRP cases, with the ORF15 exon representing the primary mutational hot-spot. Due to the highly repetitive and purine-rich sequence of ORF15, conventional short-read NGS techniques often fail to accurately detect variants in this critical region.

Material and methods: To establish an efficient diagnostic workflow for retinal dystrophies, we employed WES targeting 332 genes, including coding and hotspot non-coding variants associated with RP. Long-range PCR (LR-PCR) coupled to Sanger sequencing of the *RPGR* ORF15 was used to enhance the amplification and overcome the analysis of this problematic region, ensuring comprehensive coverage and increased diagnostic accuracy.

Results: This approach was applied to a cohort of 281 previously undiagnosed RP patients, achieving a diagnostic rate >60%. Notably, we identified causative ORF15 variants in ten patients (six males and four females), significantly improving the diagnostic yield through this method. XLRP females exhibit a milder phenotype compared to the affected males. All ORF15 variants were initially detected by WES despite at very low coverage and subsequently confirmed by LR-PCR. Among these, three were novel, contributing to the expansion of the *RPGR* mutational spectrum.

Conclusion: Our study emphasizes the need of a comprehensive molecular diagnostic approach for retinal dystrophies, combining WES with targeted Sanger sequencing to address complex genomic regions.

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P04.015.B Genetic profile of inherited retinal diseases in 497 Finnish patients

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Background: Population isolates like Finland have a unique genetic background shaped by population bottlenecks and geographical isolation, enriching certain gene variants while eliminating others. We investigated genetic findings in Finnish patients with inherited retinal diseases (IRDs).

Material and methods: We retrospectively collected data from patients with IRDs treated between 2012 and 2020 at the Helsinki University Hospital Eye Clinic. We collected the age, clinical diagnosis, mode of inheritance, and genetic findings.

Results: Of the patients who underwent genetic testing, 78% (497/639) received a likely genetic diagnosis. Most of them (90%) were native Finns. The genetic findings corresponded to autosomal recessive inheritance in 48% (239/497), X-linked in 30% (148/497), autosomal dominant in 17% (84/497), and mitochondrial inheritance in 4% (19/497) of patients. X-linked retinoschisis (*RS1*) was the most common diagnosis in 17% (83/497) of patients. The following most common causative variants were in the genes *CERKL* (8.7%, 44/497), *RPGR* (6.2 (31/497), *ABCA4* (5.4%, 27/497), *EYS* (5.2%, 26/504), *CHM* (5%, 25/497), and *CLRN1* (3.2% 16/497). *RS1*, *CERKL, CHM, EYS*, and *CLRN1* included Finnish founder mutations, explaining high variant frequencies.

Conclusion: Pathogenic variants in *RS1* (X-linked retinoschisis) and *CERKL* (cone-rod dystrophy) are the most common findings in our cohort, while Stargardt disease is less prevalent than in other Western countries. Enriched founder mutations likely contribute to the high diagnostic yield. Over one-fifth of cases remain undiagnosed, potentially due to testing limitations, non-coding variants, structural variants, or novel causative genes. Future research will leverage the Finnish population isolate to identify new IRD genes.

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P04.016.C Refining the phenotypic and genetic spectrum of oculocutaneous albinism type 8

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Background: Albinism is a heterogeneous genetic disease characterized by altered melanogenesis. To date, 21 albinism-related genes have been identified. The *DCT* gene has been associated with albinism for the first time in 2022, delineating the oculocutaneous albinism type 8 (OCA8) (PMID:33100333). Only three patients have been reported so far. Here, we present data on seven patients, including six previously unreported cases with bi-allelic variants in *DCT*, and provide detailed clinical and genetic information to enhance understanding of OCA8.

Methods: Patients with a clinical suspicion of albinism underwent targeted gene panel high-throughput sequencing. Those found to have bi-allelic variants in the *DCT* gene were included. Parental segregation was performed by Sanger sequencing. A splicing reporter minigene assay was used to assess the effect on splicing of the intronic variant c.1180-12A>G. Demographic and clinical data were retrospectively collected from geneticists, dermatologists, and ophthalmologists for each patient.

Results: Seven patients from 6 unrelated families were identified. Most exhibited no or mild cutaneous hypopigmentation and classical ocular features of albinism. All patients carried bi-allelic *DCT* variants, including missense variants, intragenic deletions, and one intronic variant. The latter altered RNA splicing according to the minigene assay.

Conclusion: Here, we describe the largest series of patients with *DCT* variants. Based on the description of seven additional patients, OCA8 appears to be primarily associated with ocular features rather than significant cutaneous hypopigmentation. This study contributes to a more precise characterization of the phenotype associated with this rare form of albinism.

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P04.017.D Homozygosity for a hypomorphic mutation in frizzled class receptor 5 causes syndromic ocular coloboma with microcornea in humans

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Background : Ocular coloboma is a congenital disorder resulting from the incomplete closure of the embryonic ocular fissure. It can arise in isolation or as part of a syndrome, exhibiting significant genetic heterogeneity. Despite advances in genetic research, the diagnostic yield remains below 30%, underscoring the need for further investigation. Mutations in the Wnt receptor FZD5, a key regulator of ocular development, have been implicated in both isolated and syndromic coloboma. These mutations often exert a dominant-negative effect, disrupting WNT signaling by sequestering WNT ligands.

Materials and methods : We describe a case of syndromic bilateral ocular coloboma with microcornea, bone anomalies, and mild intellectual disability. Whole exome sequencing identified a homozygous rare missense variant in *FZD5*. To assess its impact, we performed zebrafish embryo overexpression assays, in vitro TOPFlash assays, and in silico structural modeling.

Results: Consistent with a loss-of-function effect, overexpressing of *fzd5* mRNA harboring the missense variant in zebrafish embryos does not influence embryonic development, whereas overexpression of wild-type *fzd5* mRNA results in body axis duplications. However, in vitro TOPFlash assays revealed that the missense variant caused partial loss-of-function, behaving as a hypomorphic mutation. We further showed that the mutant protein still localized to the cell membrane and maintained proper conformation when modeled in silico, suggesting that the impairment lies in signal transduction

Conclusion : Overall, these findings mark the first reported recessive *FZD5* disease-causal variant and expand the *FZD5* disease spectrum to include syndromic complex ocular coloboma.

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P04.018.A The genetic architecture of retinal ciliopathies in the Irish Target 5000 cohort

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Background: Retinal ciliopathies (RCs) comprise syndromic and non-syndromic inherited retinal degenerations (IRDs) caused by pathogenic variants in genes required for cilia morphogenesis and function. Cilia are hair-like projections on the surface of many cell types and when defective, can cause a spectrum of clinical phenotypes including vision loss, hearing impairment, renal pathology, polydactyly, and obesity amongst others. Thus far, >100 genes have been implicated. This study aims to characterise the genetic architecture of these conditions in the Irish Target 5000 cohort.

Material and methods: Following informed consent, blood was obtained, DNA isolated according to standard protocols and next-generation sequencing undertaken. Candidate variants were interpreted according to ACMG guidelines and phased where possible. Novel missense variants were modelled using AI-powered AlphaFold and splice-altering variants of uncertain significance investigated by midigene functional assays.

Results: In total, >200 candidate variants were detected in 34 different RC-associated genes in 345 participants. Notably, 45 variants were novel. Variants of all types were identified. The most common syndromic disease was Usher syndrome followed by Bardet-Biedl syndrome, whilst variants in RPGR represented the largest number of non-syndromic IRD cases. In total, IRDs caused by RC-associated genes accounted for ~20% of solved cases in this cohort.

Conclusion: Given the clinical and genetic heterogeneity of RCs, it is of utmost importance that patients and their families obtain a molecular diagnosis in this era of precision medicine. This study illuminates the mutational profile in RCs with 45 novel variants identified and classified undoubtedly aiding the diagnostic process for future patients.

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P04.019.B Functional analysis of splicing variants located in the Proline-Serine-Threonine transactivation domain of *PAX6*

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Background: PAX6 is a transcription factor essential for ocular and neural development, whose alterations are associated with congenital aniridia. The PST transactivation domain, encoded by exons 10–13, plays a vital role in regulating gene expression. Variants altering splicing in this domain, causing exon skipping or cryptic splice sites activation, may disrupt its structure and function contributing to disease pathology. This study evaluated the spliceogenic effects of specific variants in PST-coding exons.

Material and methods: From our aniridia cohort or mutational databases, we selected variants located in PST-related exons or introns. After in silico analysis, potential spliceogenic variants were in vitro assessed through exon-trapping minigenes containing the genomic sequences of *PAX6* exons 10–13. Selected variants were introduced through site-directed mutagenesis, and both wild-type and mutated minigenes were transfected into HEK293 cells. Subsequently, splicing patterns were determined after the RNA extraction, RT-PCR and long-read sequencing with an Oxford Nanopore MinION device.

Results: Finally, fourteen variants with potential splicing effects were analysed in vitro. All variants disrupted normal splicing, resulting in complete or partial exon skipping as well as small insertions caused by the activation of cryptic exonic or intronic splice sites.

Conclusion: Splicing variants in exons 10–13 of *PAX6* can alter the structure of the PST domain. This may result in a loss or decrease of its transactivation function, potentially affecting *PAX6*-regulated pathways essential for ocular development. Therefore, in vitro minigene assays are an effective tool for evaluating the splicing impact and pathogenicity of these types of variants in aniridia.

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P04.020.C Employing a cost-effective smMIPs panel 'IRD25' to identify causative variants in known and candidate inherited retinal disease genes

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Background: Inherited retinal diseases (IRDs) are clinically and genetically heterogeneous which makes reaching a clear diagnosis challenging. Effective genetic screening is crucial to identify the causes of disease and in guiding diagnosis and treatment avenues.

Material and methods: The IRD25 panel consists of 51,245 probes targeting 358 genes associated with IRDs, SNPs associated with age-related macular degeneration, the complete *ABCA4* and *RPE65* genes, the mitochondrial genome, as well as recently published and candidate IRD genes. Probes cover all exonic regions as well as known causal intronic variants and non-coding regions of interest such as the RP17 locus. Each targeted position was covered by at least seven probes. Sequencing was performed using NovaSeq SP technology and variant analysis was performed using the Franklin by Genoox platform.

Results: A cohort of 120 IRD probands was analysed to assess the panel, and an average coverage of 294X was obtained. A 52% (83/160) of the affected individuals carried pathogenic variants (ACMG class 3-4-5) and were considered likely or possibly solved. Amongst the (likely) solved cases, the identification of deep intronic variants, variants in candidate genes, as well as potentially novel genotype-phenotype correlations highlighted the effectiveness of the panel.

Conclusion: The use of smMIPs technology to target IRD associated genes is a flexible and cost-effective approach that can be continuously updated. The IRD25 panel enables a genotype-first analysis of IRD patients that promises to bring to light novel genotype-phenotype correlations and improves the clinical diagnosis for affected individuals.

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P04.021.D Unilateral, bilateral symmetric or asymmetric isolated hearing loss in patients with heterozygous *KITLG* variants

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Oliveira[1], Serge Romana[1], Camille Porteret[9], Pierre Blanc[10], Luke Mansard[11,12], Anne Francoise Roux[11,12], Sandrine marlin, MD PhD[13,2,3], Véronique Pingault[1,14]

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Background: *KITLG* pathogenic variants have been associated to three distinct clinical presentations with different combinations of hearing loss and/or pigmentation abnormalities. However, its involvement in isolated hearing loss has not been confirmed since its initial description in two families. Besides, *KITLG* is so far the only gene prevailingly involved in unilateral isolated hearing loss.

Material and methods: We therefore conducted a retrospective study of patients with *KITLG* alterations in the French national Reference Network for Genetic Hearing Loss and one case was added through the Genematcher exchange platform.

Results: We describe a series of monoallelic *KITLG* deletions and variations in a cohort of 14 symptomatic patients from eight unrelated families. All patients presented with unilateral, bilateral symmetric or asymmetric sensorineural hearing loss. When not profound, hearing loss was predominant on low frequencies. Most *KITLG* alterations are likely to result in loss-of-function and aggregate in the extracellular region, disrupting the KIT-binding domain or its structure. Penetrance is not complete, and unspecific pigmentation alterations were observed in only three patients.

Conclusion: The present study confirms *KITLG* involvement in isolated unilateral, bilateral symmetric or asymmetric hearing loss. This confirmation indicates that genetic testing can be relevant in early-onset, non-sudden, isolated unilateral hearing loss.

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P04.022.A Long-read adaptive nanopore sequencing reveals a novel pathogenic structural variant in the *PRPH2* gene in patients with macular dystrophy

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Background: The peripherin 2 (*PRPH2*) gene is involved in photoreceptor organization. Pathogenic variants in *PRPH2* are associated with retinal and macular dystrophies with a wide degree of clinical variability. Here, we report three individuals from two families with macular dystrophy caused by exon 2 duplication of *PRPH2*.

Material and methods: Two affected members (P1, P2) from one family were treated at Helsinki University Hospital, Finland, and one unrelated individual (P3) at Loma Linda University Eye Institute, USA. The patients underwent comprehensive ophthalmic examination, and their genomic DNA was subjected to macular (P2) and retinal (P3) dystrophy gene panels or targeted genetic testing (P1). The gene panels indicated a structural variant in *PRPH2*, which was characterized by nanopore sequencing.

Results: The patients were clinically diagnosed with different forms of macular dystrophy. Exome-based genetic testing indicated all patients as heterozygous for a 482 bp duplication, covering exon 2 of *PRPH2*, with unknown breakpoints (BP). Nanopore sequencing revealed the duplication to be ~4 kb in size. They share the same 3′BP adjacent to an intronic Alu element. For P1 and P2, the 5′BP resides in a novel Alu element (AluYa5) lacking from the hg38 reference genome. P3 is negative for the novel AluYa5, and the 5′BP resides some 300 bp upstream in another known Alu element.

Conclusion: Long-read sequencing revealed that macular dystrophy in two families is caused by a 4 kb duplication involving *PRPH2* exon 2, likely due to non-allelic homologous recombination between Alu elements at the breakpoints.

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P04.024.C Screening for *SLC26A4*-related hearing loss, enlarged vestibular aqueduct and Pendred syndrome and functional characterization of novel variants in the *SLC26A4* gene

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Background: Pathogenic variants in the *SLC26A4* gene are associated with non-syndromic hearing loss with enlarged vestibular aqueduct (NSEVA) and Pendred syndrome. In the Caucasian population, the CEVA haplotype plays an important role in diagnostics of NSEVA. The aim of the study was identification of genetic etiology in 37 probands with hearing loss, EVA or Pendred syndrome and functional characterization of novel variants in the *SLC26A4* gene.

Material and methods: For determination of genetic etiology, Sanger sequencing, WES and KASP genotyping assay were used. The Nanopore sequencing of nasopharyngeal swab samples was performed to confirm the pathogenic effect of potential splice site variant. Functional characterization of *SLC26A4* variants involved determination of iodide influx, total and plasma membrane pendrin expression level and subcellular localization of pendrin by confocal imaging.

Results: Biallelic variants in the *SLC26A4* gene were identified in 10 probands and a complete CEVA haplotype was confirmed in three probands harbouring *SLC26A4* monoallelic variants. Sixteen variants in the *SLC26A4* gene were identified in total, three of which are novel. The functional characterization of novel variants and variants which were not functionally characterized yet confirmed pathogenic potential of five out of six tested variants. Analysis of nasopharyngeal swab samples confirmed exon 4 skipping due to novel variant *SLC26A4*:c.415G>A.

Conclusion: The genetic etiology of hearing loss associated with EVA was determined in 13 out of 37 probands (35%). Functional studies are important for the accurate diagnosis, as well as for a better understanding of the function of *SLC26A4* variants.

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P04.025.D Exploring the genetic background of autosomal dominant hearing loss

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Introduction: Autosomal dominant hearing loss (ADHL) is the second most common form of hereditary HL, typically manifesting after the first decade of life. It primarily affects high frequencies and worsens over time. Autosomal-dominant genes account for about 20% of prelingual non-syndromic deafness cases, with 63 genes identified to date.

Materials and methods:

This study included 110 ADHL families. Targeted next-generation sequencing (NGS) of 237 HL-related genes was performed in all probands. In six large unsolved families, linkage analysis and whole genome sequencing (WGS) were conducted. Presence of the selected probably pathogenic variants and their segregation with HL within the family were confirmed by standard Sanger sequencing.

Results: A genetic cause was identified in 51% (56/110) of families. Among 56 HL variants, 27% (15/56) were previously reported, while 73% (41/56) were novel. Variants included missense (35/56, 62%), splice site (8/56, 14%), frameshift (6/56, 11%), nonsense (6/56, 11%), and one synonymous variant (1/56, 2%). The most frequently affected genes were *MYO6* (n = 11), *TBC1D24* (n = 6), *WFS1* (n = 6), *GSDME* (n = 5), *POU4F3* (n = 5), and *KCNQ4* (n = 4). Variants in *NLRP3*, *LMX1A*, *FGFR3*, *CD164*, *GRHL2*, *TMC1*, *COCH*, *ATP2B2*, and *CEACAM16* were found in single families. Linkage analysis and WGS identified non-coding variants in *EYA4* and *ATP11A* and novel candidate genes.

Conclusions:

Our custom multigene panel demonstrated good diagnostic performance. Given the frequent discovery of novel variants, clinical assessment and segregation analysis are essential. Linkage analysis and WGS improve variant detection, particularly in non-coding regions, and help identify new HL genes.

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P04.026.A Identification of novel candidate genes for inherited retinal disorders by studying 609 consanguineous families

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Background: Consanguinity provides a unique opportunity to identify novel gene-phenotype correlations. Consanguineous children have an average genomic homozygosity 10-fold higher than outbred individuals. This facilitates the identification of high-impact genomic variants for autosomal recessive inherited retinal disorders (IRDs). Pakistan and Iran are among the most consanguineous countries in the world, with consanguineous marriages accounting for up to 70% and 38%, respectively. The purpose of this study was to identify novel candidate genes for IRDs by studying consanguineous families.

Methods: This study presents the genetic analysis of 609 consanguineous multiplex families from Pakistan and Iran. WES was performed on the affected individuals, and in some unsolved families, SNP-arrays were used for homozygosity mapping.

Results: Overall, the diagnostic yield was ~70%. The most frequently implicated genes were CRB1 (18-families), AIPL1 (11-families), and ABCA4 (11-families). In addition, pathogenic variants were identified in LCA5 (10-families), RPE65 (7-families), RPGR (3-families), MYO7A (3-families), CNGA3 (7-families), and CNGB3 (3-families), all of which are treatable targets for gene therapy that is either currently available or undergoing clinical investigation. Among the undiagnosed families, likely pathogenic variants in 37 novel candidate genes were identified, and these findings will be presented at the conference.

Conclusion: This study demonstrates the power of investigating consanguineous multiplex families to identify novel genes,

thereby expanding our understanding of the molecular pathways underlying IRDs. These discoveries are of paramount clinical importance not only for specific families or geo-ethnic groups, but also for patients worldwide, as they improve diagnostic possibilities and therapeutic interventions in diverse clinical settings.

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P04.027.B Major impact of *USH2A* variants on the development of mild to moderate hearing loss

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Background: The most common genetic cause of mild to moderate hearing loss (HL) is *STRC* copy number variants (CNVs). Many patients with mild to moderate HL require further genetic testing due to the lack of molecular diagnosis. Literature indicates that, besides *STRC, USH2A, MPZL2*, and *OTOG* may also be responsible for mild to moderate HL. This study aimed to identify the genetic basis of isolated mild to moderate HL in patients without *STRC* CNVs.

Material and methods: The study included 47 patients with isolated mild to moderate HL without detected *STRC* CNVs or with one identified heterozygous *STRC* CNV. Genomic DNA was isolated from peripheral blood and NGS with a custom panel of HL-related genes (n = 237) was performed. Selected variants were confirmed by Sanger sequencing.

Results: NGS revealed the genetic cause of HL in 51% (24/47) of patients. Among these, 25% (6/24) of causative variants were found in *USH2A*, linked to Usher syndrome. In 17% (4/24) of variants, mutations in *OTOG* were considered as the cause of HL. The remaining causative variants were detected in: *ADGRV1, GATA3, MPZL2, OTOA, POU3F4, PTPRQ, REST, STRC, TBC1D24*, and *WFS1*.

Conclusions: NGS revealed that, in addition to *STRC*, a significant proportion of patients with mild to moderate HL have causative variants in *USH2A*. Patients with *USH2A* pathogenic variants are likely to develop Usher syndrome type 2A, which includes both HL and retinitis pigmentosa. Identifying the genetic cause is crucial for providing personalized care from an early age.

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P04.028.C 2D and 3D human in vitro models: a platform to explore the impact of SOX2 hemizygosity on nervous system development

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Background: Syndromic microphthalmia-3 (MCOPS3, #206900) is a rare disorder, characterized by anophthalmia microphthalmia and esophageal atresia. Genital anomalies are common in male patients associated with central nervous system developmental abnormalities, cardiac defects, vertebral anomalies, growth failure, sensorineural hearing loss, pituitary hypoplasia, and hypogonadotropic hypogonadism. Intellectual development is very variable. MCOPS3 is an autosomal dominant disorder, due to mutations in the SOX2 gene (MIM#184429), which plays a crucial role in nervous system development and stem cell maintenance.

Material and ethods: induced pluripotent stem cells (iPSCs) were generated using Sendai virus from peripheral blood of 2 patients heterozygous for a SOX2 truncating variant, respectively NM_003106.4:c.70_89del (case 1 with right anophthalmia, left microphthalmia, and neurological symptoms) and NM_003106.4:c.53C>A (case 2 with bilateral anophthalmia/microphthalmia, hypogenitalism, and epilepsy). Using CRISPR/Cas9 isogenic clones were generated from case 2's IPSCs.

Results of the iPSCs from both cases properly expressed stemness markers. Cytofluorimetry in the IPSCS proved a reduced SOX2 expression when compared with healthy controls and isogenic edited clones. Retinal Ganglion cells(RGCs), cortical neuronal cells, and cortical organoids were differentiated by specific protocols. Immunofluorescence and RT-qPCR confirmed the cellular identity of the models. We are defining the genomic pattern of SOX2 binding to DNA by CUT&RUN, to identify targets for which SOX2 dosage reduction results in reduced or lost binding, plausibly relevant for SOX2-dependent pathology.

Conclusion: We proved that the haploinsufficiency of SOX2 does not preclude obtaining iPSCs and maintaining their stemness. For the first time, we generated human 2D and 3D models with heterozygous SOX2 variants.

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P04.029.D Identification of the RPGR gene pathogenic variants in a cohort of Greek male patients with retinitis pigmentosa phenotype

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Background: X-linked Retinitis Pigmentosa (XLRP) is one of the most severe retinal phenotypes accounting for about 20% of all RP patients. RPGR is the most commonly implicated gene and its variants account for more than 70% of XLRP. The aim of the study was to report the molecular basis of RPGR related retinitis pigmentosa in 16 Greek unrelated patients.

Material and ethods: Genetic testing of 441 independent Greek patients with suspected retinal dystrophies was performed using a Next Generation Sequencing panel of retinal dystrophy genes as described (PMID: 34985506).

Results: The molecular background was established in 374 patients of the total 441 tested. RPGR pathogenic variants were detected in 16 unrelated, male patients (4.27%) in this cohort.

Demographic data including family history and reported diagnosis were available. 10 patients were simplex, 3 had an affected brother, 1 had an affected sister and 2 had numerous male and female affected relatives resembling an autosomal dominant inheritance. Variants in ORF15 accounted for 68.75% (11/16) of the diagnoses, the remaining variants were detected in exons 2 and 9 (missense), 4 and 9 (frameshift) whereas a deletion of exons 1–11 of the RPGR gene was detected in 1 case.

Conclusion: This study provides the first molecular genetic characteristics of patients with RPGR pathogenic variants from the previously unexplored Greek population. Our study expands the mutational spectrum as we report novel variants in the RPGR gene and allows for the formation of a cohort for current and emerging therapeutic opportunities for this disorder.

C.O.I. Disclosure:

S. Kamakari: None. **S. Koukoula:** None.

P04.030.A Novel genetics findings using WES and WGS and implications of these in optic nerve atrophy

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Background: Optic atrophy is a severe eye condition in which the retinal ganglion cells degenerate, causing vision loss and a pale optic nerve when visualized though fundoscopy. Optic atrophy can be genetic; Autosomal dominant optic atrophy (ADOA) is caused by pathogenic variants in *OPA1*, and Leber's hereditary optic neuropathy (LHON) is caused by specific pathogenic mitochondrial variants. More rare genetic causes of optic atrophy may be uncovered using non-biased genetic testing, such as whole exome/genome sequencing (WES/WGS). Uncovering the genetic cause can have great implications for patients and families, including more knowledge about the expected visual prognosis, possible extraocular manifestations, and the hereditary mode. Here, we investigated a cohort of patients with optic atrophy with WES/WGS to estimate the diagnostic yield of unbiased genetic testing in optic atrophy and uncover more uncommon causes.

Material and methods: WES or WGS analysis in patients with optic atrophy.

Results: 68 patients had WGS (15) or WES (53) performed. 22/68 had a genetic diagnosis (yield 32%). Genetic findings included *OPA1* (50%) but also rare variants in genes such as *WFS1* (9,3%), *NR2F1* (6,2%), and *ACO2* (6,2%). For 22 patients the genetic finding implicated more than optic atrophy, e.g., intellectual disability (*NR2F1, ACO2, COQ2)*, hearing loss (*NR2F1, ACO2, COQ2, WFS1*) and other features (*UCHL1, COQ2, WFS1*).

Conclusion: This study illustrates that un-biased genetic testing have clinical implications in patients with optic atrophy that are not limited to ADOA and LHON but includes other rare genetic causes that may have diagnostic implications.

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P04.031.B Single-center experience on genotype-phenotype correlation in retinitis pigmentosa cases in the Marmara Region

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Background: Retinitis pigmentosa (RP) is a genetically heterogeneous retinal disorder characterized by progressive vision loss. Next-generation sequencing (NGS) is essential for identifying causative mutations, establishing genotype-phenotype correlations, and improving clinical management. This study aims to characterize the genetic spectrum of RP in the Turkish population using NGS.

Material and methods: A total of 118 unrelated Turkish RP patients were recruited at Bursa Uludag University Hospital, and clinical or whole-exome sequencing was performed using the Illumina NextSeq system, with variants classified per ACMG guidelines. Clinical evaluations, including fundoscopic examination, OCT, ERG, and visual field analysis, were conducted, and statistical analyses assessed genotype-phenotype correlations.

Results: Pathogenic or likely pathogenic mutations were detected in 94 patients, spanning 48 genes, with a total of 84 mutations, including 16 novel variants. Clinically relevant variants were identified mostly in *MYO7A* (n = 7), *CDHR1* (n = 6), *NR2E3* (n = 5), *RDH12* (n = 5), *RPGR* (n = 5), and *USH2A* (n = 5). Mutation inheritance patterns included 61 homozygous, 19 heterozygous, 10 compound heterozygous, and 4 hemizygous variants. According to ACMG criteria, among the 84 identified variants, 37 were pathogenic, 21 likely pathogenic, and 26 variants of uncertain significance (VUS). *MYO7A* mutations were associated with earlier disease onset ($P = 0.001$) and higher cystoid macular edema frequency in the right eye ($P = 0.021$) compared to *CDHR1* mutations.

Conclusion: This study provides the most comprehensive multidisciplinary molecular analysis of RP in the Turkish population, identifying 16 novel variants and expanding the known RP mutation spectrum. The high diagnostic yield of 79.6% underscores the clinical value of NGS-based molecular testing for RP.

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P04.032.C Identification of susceptible mutations and digenic pathogenic combinations of Retinitis Pigmentosa in Chinese families cohort

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Background: Retinitis pigmentosa (RP; MIM: #268000) is a group of inherited retinal dystrophies caused by the loss of retinal photoreceptors. It is a clinically and genetically heterogeneous disorder. Understanding the genetic mutations and their combinations can aid in identifying pathogenic mechanisms and improving diagnosis for RP.

Material and methods: The study analyzed 339 trios of RP families using genetic analysis, including the Transmission Disequilibrium Test (TDT). Bonferroni correction was applied to identify significant mutations. ORVAL software was utilized to predict the pathogenicity of digenic combinations of mutations. Statistical, machine learning, and genetic methods were employed to analyze and validate the findings.

Results: Seven significant mutations associated with RP were identified after Bonferroni correction: *MSH2* c.212-16delT, *LRP5* c.58_60del, *FOXE3* c.211A>G, *PRX* c.4077_4079del, *DMD* c.8810A>A, *GP1BA* c.1322_1344del, *SYNE2* c.1170A>C, and *SMPD1* c.573T>C. In addition, 26 combinations of highly correlated susceptible loci were identified, involving 31 genes. Two digenic

combinations were predicted as pathogenic: *COL3A1* c.2092G>A~*TWIST1* c.295A>G and *CNNM4~WFS1*. The *COL3A1* c.2092G>A~*TWIST1* c.295A>G combination was classified within the Disease Causing 99% zone and could explain the RP phenotype in four families.

Conclusion: The study identified *COL3A1* c.2092G>A~*TWIST1* c.295A>G as a novel digenic combination associated with RP, demonstrating the utility of integrating statistical, machine learning, and genetic methods in uncovering pathogenic genetic interactions. These findings provide new insights into the genetic basis of RP and potential targets for diagnosis and treatment.

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Z. Wang: None. **D. Han:** None. **X. Chen:** None.

P04.033.D Identification of a novel heterozygous OPA3 mutation in a large Italian family with cataract and progressive sensorineural deafness

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Background: Co-occurrence of cataracts and sensorineural hearing loss in the young was initially documented by Nadol and Burgess in 1982 in a large family (MIM120040). We described a second large Italian family including 11 affected members spanning 4 generations, with a similar phenotype (PMID:1623624).

Aim: To identify the genetic cause in the Italian family with cataract and sensorineural hearing loss syndrome.

Material and methods: WES and SNP array was performed on 11 family members on 4 generations, including both healthy (N = 5) members and those with syndrome (N = 6). Ten linkage regions (LOD>1) were identified. WGS was performed on one affected member to investigate the linkage regions for structural variants, tandem repeats, and non-coding regions.

Results: We found a novel heterozygous missense mutation (p.Lys10Asn, chr19:46087993 C>A, hg19) in OPA3 gene, segregating in all family members. OPA3 mutations are associated with ADOAC (autosomal dominant optic atrophy and cataract), whose clinical manifestations overlap with our patients except for the absence of optic atrophy. Notably, another research group reported the same mutation in a separate family with the same peculiar clinical presentation as ours (https://doi.org/10.1002/ajmg.a.63846).

Conclusion: We found the likely genetic cause of a rare syndrome in a large Italian family, a missense mutation in OPA3. Dominant mutations in OPA3 cause ADOAC, whose clinical manifestations partially overlap with our affected family members, despite the absence of optic atrophy. Interestingly, another group found the same mutation in a second family with same symptoms, suggesting that our finding could expand the spectrum of OPA3-associated diseases.

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P04.034.A Ophthalmic genetics service in a tertiary center: diagnostic yield, insights and challenges

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Background: Ophthalmic genetics is a rapidly evolving field, with over 600 genes implicated in inherited eye disorders in the past three decades. The growing accessibility of Next Generation Sequencing (NGS) technology has revolutionized this field, shortening the diagnostic odyssey for countless pediatric and adult patients. The diagnostic yield of NGS-based genetic testing in patients with ophthalmological conditions suspected to have a monogenic etiology varies greatly depending on the phenotype.

Methods: We established a referral ophthalmo-genetics clinic in a large tertiary center, led by a single dedicated genetic counsellor, supervised by a medical geneticist. We sought to characterize the patient population and evaluate the overall and phenotype-specific diagnostic yield of NGS-based genetic testing.

Results: Between 2021–2023, a total of 141 unrelated probands (age range, 2.5 weeks to 84 years) were evaluated at the Ophthalmic Genetics Clinic, 73 (51.8%) of whom under 18 years of age. The most common clinical presentations were Retinitis Pigmentosa and suspected hereditary retinopathies/viteroretinopathies (37.6%), followed by high myopia (13.5%), congenital cataract (9.9%) and suspected ocular albinism (9.2%). Fourty-three probands (30.5%) completed at least one comprehensive genetic test (most often, gene panel sequencing) by the time of analysis. Of these, the overall diagnostic yield was 51.2% (22/43), with an additional 32.5% (14/43) cases in which genetic testing resulted in variants of uncertain significance (VUS). Diagnostic yield differed between clinical presentations.

Conclusions: Our findings underscore the importance of NGS-based genetic testing in individuals with suspected inherited eye disorders. Furthermore, we present a cost-effective model for a referral ophthalmo-genetics clinic.

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P04.035.B Screening of the DNAJC30 gene causing autosomal recessive Leber hereditary optic neuropathy in a Finnish cohort

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Background: Leber hereditary optic neuropathy (LHON) is a rare mitochondrial disease characterized by vision loss, typically manifesting in the second or third decade of life. While most cases of mitochondrial LHON are associated with three common mitochondrial DNA variants, autosomal recessive LHON (arLHON) has been recently linked to biallelic variants in the DNAJC30 gene. DNAJC30 has a gene carrier frequency of 1% in the Finnish population, mainly caused by the NM_032317.3(DNAJC30):-c.152A>G p.(Tyr51Cys) variant. Notably, the Finnish population has the highest allele frequency of this variant in gnomAD v4.1.0 and 3.1.2.

Material and methods: As part of the R&D of a clinical laboratory test we screened anonymized Finnish patients, who had tested negative for mitochondrial LHON point mutation analysis during 2004–2024, for the NM_032317.3(DNAJC30):-c.152A>G p.(Tyr51Cys) variant. This variant site was analyzed by Sanger sequencing in total 133 patients from the Helsinki University Hospital.

Results: We identified three DNAJC30 c.152A>G p.(Tyr51Cys) homozygotes and three heterozygotes. Using the Hardy-Weinberg equation, gnomAD allele frequencies and population of 5.6 million, we estimated that 100 Finns would have the c.152A>G p.(Tyr51Cys) variant in homozygous state at population level. FinnGen genotyping data from 500,348 Finnish individuals shows 14 homozygotes and in recessive analysis, association with binocular visual impairment was observed (p-value = 2.34e-4).

Conclusion: A recent study reporting two Estonian arLHON cases hypotized that this variants prevalence would be even higher among Finns. We thereby expected to find more homozygotes. However, DNAJC30 c.152A>G p.(Tyr51Cys) does not seem to be very common cause of LHON in Finnish patients.

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P04.036.C Molecular diagnosis of hereditary optic neuropathy : retrospective next-generation sequencing study in 51 cases

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Background: Hereditary optic neuropathy is best known for its two most common phenotypes; autosomal dominant optic atrophy and Leber hereditary optic neuropathy. These disorders belong to a group of diseases arising from mitochondrial dysfunction caused by defects in both the nuclear and mitochondrial genomes.

Material and methods: We present a retrospective evaluation of 51 patients assessed between 2022–2024 with a diagnosis of hereditary optic neuropathy. 38 were male, and 13 were female, with an average age of 31(range:3–61 years). We performed clinical exome sequencing to evaluate nuclear and mitochondrial genomic alterations.

Results: Five patients were identified with pathogenic or clinically uncertain homoplasmic genomic changes in the mitochondrial genome. Two of these patients were female, and three were male, with an average age of 27 years. Consistent with the literature, the most frequently detected mitochondrial variant was m.11778 (MTND4).Pathogenic/likely pathogenic or clinically uncertain (VUS) changes in the nuclear genome were identified in 24 patients. The most frequently detected genomic alterations were found in the *OPA1* gene(14 patients) and the *WFS1* gene(3 patients). Other genes in which nuclear genomic alterations were identified included *LRP5*, *KCNJ13 NDUFAF5*, *EYS*, and *NXNL1*. Three patients had retinal diseases in addition to optic nerve disease. Retinal dystrophy-related variants were identified in two patients, specifically in *EYS* and *LRP5*.

Conclusion: In our study evaluating patients with optic neuropathy, nuclear genomic alterations were more frequently detected. However, we recommend a comprehensive assessment of both the nuclear and mitochondrial genomes in these patients.

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F.Z. Yalçin: None. **H. Erdem:** None. **H. Saat:** None. **A. Sezer:** None. **A. Bakir:** None. **E. Tasdelen:** None.

P04.037.D Identification of the genetic background of Polish pediatric patients with suspected oculocutaneous albinism using next-generation sequencing

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Background: Albinism is a phenotypically and genetically heterogeneous condition characterized by absent or reduced pigmentation of the skin, hair, eyes. Seven genes are associated with different types of autosomal recessive OCA (*DCT, LRMDA, OCA2, SLC45A2, SLC24A5 TYR, TYRP1*) and *GPR143* gene is associated with X-linked OA.

Material and methods: We analyzed 25 Polish pediatric children from unrelated families with clinical suspicion of OCA/OA, searching for molecular changes in the causative genes using NGS based multi-gene targeted panel.

Results: Molecular analysis provides a genetic diagnosis in approximately 52% of individuals with clinical OA/OCA. The most commonly mutated gene was *TYR*, associated with OCA1. In this group we found common functional polymorphisms p.(Ser192Tyr) and p.(Arg402Gln) as pathogenic haplotype, which segregates consistently with an autosomal recessive inheritance pattern in various configurations with other pathogenic variants i.e.: one novel: p.(Asp305Glufs*12) and five known p.(Arg217Gln), p.(Pro406Leu), p.(His367Gln), p.(Met1Val), p.(Thr373Lys). The remaining patients had pathogenic variants in genes: *OCA2, GPR143, SLC24A5, SLC45A2* and *TYRP1*, among them the novel ones were: *TYRP1*: p.(Lys141Profs*38); *OCA2*: p.(Ala433Val); *SLC24A5*: p.(Met114Ile). Three patients were heterozygous for a causative variation in *OCA2* or *SLC45A2*.

Conclusion: Haplotype formed by two common, functionally-relevant variants in *TYR*: p.(Ser192Tyr) and p.(Arg402Gln) is associated with a high probability of receiving an albinism diagnosis. Reporting this variants in individuals in whom only a single deleterious *TYR* variant has been identified could permit a uplift in confirmatory molecular diagnoses. The combined analysis of rare and common variants can increase diagnostic yield and enables genetic counselling in families with albinism.

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P04.038.B Congenital cataract in two unrelated individuals explained by *CRYBB2* gene conversion

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Background: Congenital cataract are opacities of the lens that develop within the first year of life. A genetic cause is found in around 50% of cases. Among about 50 causative genes described, with various modes of inheritance, those encoding crystallins are frequently involved. This includes *CRYBB2* gene, whose monoallelic pathogenic variants are associated with autosomal dominant isolated congenital cataracts. We report herein two individuals with a conversion involving *CRYBB2* and its pseudogene.

Materials and methods: Two unrelated individuals (a 4-month-old female and a 38-year-old female) with isolated congenital cataract were analysed using a targeted panel of 187 ocular development genes. An exon 5 deletion of *CRYBB2* was suspected and further investigated using custom CGHa (180 k) and long-range PCR.

Results: A heterozygous deletion of exon 5 of the *CRYBB2* gene (NM_000496.2) was suspected in both individuals but not confirmed by high resolution CGHa. Gene-specific Long-Range amplification and Sanger sequencing revealed three heterozygous missense variants in exon 5 of *CRYBB2*: p.(Arg145Trp), p.(Gln147Arg) and p.(Thr150Met). These variations matched the reference sequence of the pseudogene *CRYBB2P1* exon 4, supporting a conversion event between exon 5 of *CRYBB2* and exon 4 of *CRYBB2P1*.

Conclusion: Molecular studies in these patients identified a gene conversion involving *CRYBB2*. Such *CRYBB2* gene conversions, previously described in 3 patients with congenital cataract, may explain part of patients without molecular diagnosis as they could easily be missed by short-read sequencing. Recognizing gene conversion mechanisms is crucial for improving molecular diagnoses, as other genes may also be affected by similar events.

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P04.039.A Genetic analysis of 387 independent otosclerosis patients

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Backround: Otosclerosis is a disease affecting the middle and inner ear, which manifests in a slowly developing, intermittent hearing loss eventually leading to deafness. Hearing loss increases as the transmission of sound via the eardrum and ossicular chain is increasingly impaired because of inflammation-like remodelling processes of the bone with fixation of the stapes footplate to the oval window caused by genetic, viral and hormonal influences. Three different types of Otosclerosis can be distinguished. The classic middle ear type with pure conductive hearing loss. The pure inner ear type, capsular Otosclerosis with sensorineural hearing loss. The affection of both the middle and inner ear with a combination of conductive and sensorineural hearing loss. So far, eight different gene locations for Otosclerosis have been identified in single families and four genes that have so far only been described in individual patients. However, a clearly responsible gene has not been idetified yet.

Material and methods: The aim of the research project is to identify genes responsible for Otosclerosis in a larger sample collection of around 387 independent clinically clearly characterized patients. DNA sequencing techniques and Q-PCR methods will be used to analyse whether the genes FOXL1, MEPE, SMARCA4 and SERPINF1 are involved in Otosclerosis.

Results: The investigated samples do not show any mutations in the FOXL1 gene, no alterations were detected in MEPE so far.

Conclusion: In contrast to literature, involvement of the FOXL1 gene in Otosclerosis was excluded, while the impacts of the MEPE gene cannot be conclusively assessed yet.

C.O.I. Disclosure:

L. Nagel: None. **S. Hollander:** None. **S. Amann:** None. **R. Birkenhager:** None.

P04.040.C Gene variant spectrum in a cohort of 94 patients with familial exudative vitreoretinopathy

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Background: Familial exudative vitreoretinopathy (FEVR) is a rare genetic disease that impedes the vascularization of the retina. Early treatment before the development of complications is critical for the prevention of vision loss in moderate to severe cases. For this, a genetic diagnosis is essential, however, only 50% of FEVR patients have a variant in a known FEVR gene. Over 25 genes have been associated with the FEVR phenotype, with most cases being in *FZD4*, *LRP5*, *TSPAN12*, and *NDP*.

Material and methods: Probands clinically diagnosed with FEVR were enrolled and clinical information and DNA collected. An expanded FEVR panel was used, including six recognized FEVR genes (*FZD4, NDP, LRP5*, *TSPAN12*, *ZNF408*, and *CTNNB1*) and 19 genes previously associated with ocular features overlapping FEVR (FEVR-associated genes). Variants identified using targeted next-generation sequencing were analyzed and classified using the ACMG and ClinGen SVI working group recommendations to detect disease-causing variants (DCV).

Results: Analyses of data from a cohort of 94 probands provided a molecular diagnosis for 40 (42.6%) probands: 35 (87.5%) had a single DCV while five (12.5%) harboured more than one. Of 42 total DCV, 33 (78.6%) were in four of the six recognized genes (*LRP5*, *FZD4*, *TSPAN12*, *NDP*) while eight were found in FEVR-associated genes—six in *KIF11* and two in in association with another DCV.

Conclusion: The expanded FEVR gene panel detected DCV in nearly half of our cohort. Four FEVR genes account for most cases, and the role of rare FEVR genes and candidate genes requires further study.

C.O.I. Disclosure:

S.R. van der Ende: None. **C.R. McMaster:** None. **J.M. Robitaille:** None.

P04.041.D Identification of two pathogenic splice variants, in the *HPS5* gene, associated with Hermansky-Pudlak Syndrome 5

Francesca Peluso[1], *Neri Alberto*[1], *Niki Ionadi*[1], *Isabelle Bacchi*[2], *Stefano Giuseppe Caraffi, PhD*[3], *Chiara Cattani*[4], *Roberta Zuntini*[3], *Livia Garavelli*[5]

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Background: The *HPS5* gene codes for two isoforms: isoform A (NM_181507.2) (full-length), which consists of 23 exons encoding

a protein of 1129 amino acids, while isoform B (NM_007216.4) has a different 5' UTR and lacks exon 2, resulting in the usage of downstream ATG and in a protein that lacks the first 114 amino acids. Pathogenic variants, in the *HPS5* gene, are associated with Hermansky-Pudlak syndrome 5, which causes oculocutaneous albinism and a bleeding diathesis due to dysfunction of lysosome-related organelles (RLOs) and absence of dense platelet bodies.

Material and method: Whole exome sequencing filtered for genes associated with oculocutaneous albinism was performed. In vitro RNA analysis was carried out to clarify the effects of variants on splicing.

Results: We detected two new variants in three probands with oculocutaneous albinism. The first two probands are two sisters of consanguineous Pakistani parents. A homozygous variant was identified in the *HPS5* gene considering isoform A as reference, (NM_181507.2):c.108G>A that does not result in amino acid change p.(Lys36=). The third proband, a girl from a non-consanguineous Italian family, was compound heterozygous for the known pathogenic variant c.543del, inherited from her father, and for the novel variant c.1784+1G>A inherited from her mother. In the three patients, haematochemical examinations were normal and they have no history of bleeding problems. RNA analysis was carried out for both variants and revealed transcript alterations that can explain the clinical presentation.

Conclusion: We have identified two new splicing variants that cause a milder haematological phenotype of Hermansky-Pudlak syndrome 5.

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P04.042.A Genotype and phenotype characteristics of patients with familial exudative vitreoretinopathy: first report on a Turkish cohort

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Background: We aimed to investigate genetic and clinical characteristics of patients with familial exudative vitreoretinopathy (FEVR) in a large single-center Turkish cohort.

Material and methods: The genetic test results of 95 patients diagnosed with FEVR in the ophthalmology department of Gazi Medical Faculty were retrospectively evaluated. The reported gene variants and clinical findings were recorded. Clinical severity was evaluated in patients with a reported variant in known FEVR genes (L*RP5, FZD4, NDP, TSPAN12, ZNF408, KIF11, DOCK6*, and *CTNNB1*).

Results: The genetic test results were conclusive in 33 patients (34.7%) with pathogenic/likely pathogenic variants, inconclusive for 17 patients (17.9%) with variants of uncertain significance (VUS), and negative for the remaining patients (47.4%) with no variants in known FEVR genes. Forty-five variants were reported in 38 unrelated patients, 19 of them (42.2%) were novel. *LRP5* variants were most prevalent (16/38), followed by *FZD4* (6/38), *TSPAN12* (5/38), *CTNNB1* (3/38), *NDP* (2/38), *KIF11* (2/38), *ZNF408* (1/38), and *DOCK6* (1/38). Two patients had more than one variant in more than one FEVR gene. Asymmetry was most prevalent in patients with *FZD4* variants (50%). However, patients with *LRP5* (37,5%), *TSPAN12, ZNF408*, and *CTNNB1* variants also showed asymmetric eye findings. Our cohort included six families with more than two patients, and four had younger family members with more severe findings.

Conclusion: This study represents one of the largest cohorts from Europe and the first cohort from Turkey investigating genotype-phenotype characteristics in FEVR patients. Our study supported the phenotypic variability in FEVR, even in the same family members.

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P04.043.B Newly described *LRP2* pathogenic variants in a patient with Donnai-Barrow syndrome without congenital malformations

Sara Bargiacchi[1], *Lucia Tiberi*[1], *Giacomo M. Bacci*[2], *Francesco Marco Parodo*[3], *Chiara Quisisana*[4], *Roberto Caputo*[2], *Debora Vergani*[1], *Elia Dirupo*[1], *Ilaria Sani*[1], *Marilena Pantaleo*[1], ***Rosangela Artuso***[1], *Angela Peron*[1,3]

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Background: Donnai-Barrow syndrome (DBS), also known as facio-oculo-acoustico-renal (FOAR) syndrome, is characterized by multiple congenital malformations with distinctive facial features, myopia and other ocular findings, hearing loss, agenesis of the corpus callosum, low-molecular-weight proteinuria and variable intellectual disability. Congenital diaphragmatic hernia (CDH) and/or omphalocele are common. Fewer than 60 individuals from about 20 families have been reported. DBS is an autosomal recessive disorder caused by loss of function variants in *LRP2* (low-density lipoprotein receptor-related protein 2 gene, chr 2q31.1) encoding the protein megalin, expressed in multiple absorptive epithelia, notably in the brain, kidney, and eye.

Material and methods: Few reports in the literature mention that a milder phenotype was observed in some patients. Here we describe a 6-years-old patient with a phenotype characterized only by high myopia, esotropia and minimal behavioral differences. We also describe the ocular phenotype in detail.

Results: On trio exome sequencing, we identified two compound heterozygous pathogenic variants in *LRP2*: a missense variation, c.9014G>A (p.Cys3005Tyr), and a deletion involving the entire gene. Both variants have not been previously reported in literature and entire gene deletions, in particular, have not been described thus far in association with DBS.

Conclusion: We describe the detailed phenotype of a patient with DBS showing no congenital malformations and we report for the first time a deletion of the entire *LRP2* gene. This information helps better understand genotype-phenotype correlations of this rare condition.

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P04.044.C Genetic background of inner ear malformations

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Background: Inner ear malformations (IEMs) can occur in isolation or as part of genetically determined syndromes. Enlarged vestibular aqueduct (EVA) is the most frequently observed IEM, often accompanied by incomplete partition type 2 (IP2) of the cochlea and a dilated vestibule. *SLC26A4* is the major gene associated with IEMs, with 50% of EVA/IP2 patients carrying either two pathogenic *SLC26A4* variants or one *SLC26A4* pathogenic variant together with the CEVA haplotype. The genetic background of other IEMs is more diverse and remains poorly understood.

Material and methods: DNA was isolated from blood samples of 40 hearing loss (HL) patients with bilateral IEMs. Sequencing was performed using a custom multigene panel (237 or 263 genes).

Results: Genetic testing identified the cause of IEMs in 67.5% (27/40) of individuals. Among EVA/IP2 patients, six carried two pathogenic variants in *SLC26A4*, while another six had one *SLC26A4* pathogenic variant together with the CEVA haplotype. The genetic cause still remains unknown in six EVA/IP2 patients. In patients with other IEMs, pathogenic variants were identified in *POU3F4* as well as genes associated with CHARGE (*CHD7*), BOR (*EYA1*, *SIX1*), Waardenburg (*SOX10*), and Kabuki (*KMT2D*) syndromes. Additionally, a novel pathogenic variant in *HOXA2* was identified in a single patient.

Conclusion: Mutations in *SLC26A4* and the CEVA haplotype account for more than half of EVA/IP2 cases. Patients with other IEMs are frequently diagnosed with syndromic forms of HL. Further research is needed to identify pathogenic variants in noncoding regions of known HL genes or in novel genes.

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P04.045.D The burden of combined sensory impairment and two distinct genetic diseases in a single patient

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Background: Waardenburg syndrome refers to a group of rare genetic disorders characterized by congenital sensorineural hearing loss (SNHL) and defects in neural crest-derived structures, including pigmentation anomalies of the eyes, hair and skin. Waardenburg syndrome type 1 (WS1, MIM#193500) is the second most common subtype and is distinguished by eye dysmorphology, such as dystopia canthorum, which is typically not associated with visual impairment. This case presents the concomitance of WS1 and Stargardt 1 disease or Retinitis Pigmentosa (RP).

Material and methods: A 6-year-old boy with unilateral, *GJB2*-negative SNHL and a family history suggestive of autosomal dominant inheritance was referred for genetic evaluation. His phenotype was consistent with WS1, exhibiting four major and two minor criteria. Whole Exome Sequencing (WES) was performed to confirm the clinical diagnosis.

Results: WES confirmed WS1 diagnosis, identifying a novel likely pathogenic variant in *PAX3* [NM_181459.4:c.688C>A, p.(Gln230Lys)]. Additionally, compound heterozygosity was detected in *ABCA4* [NM_000350.3:c.5882G>A, p.(Gly1961Glu)/NM_000350.3:c.5917del, p.(Val1973*)]. Both of these variants are known causative for Stargardt 1 (MIM#248200) or RP (MIM#601718).

Conclusion: This case underscores the value of WES in revealing both expected and incidental findings. The patient's congenital SNHL is compounded by the risk of future visual impairment, which will significantly affect his quality of life, particularly in communication and social integration. Genetic diagnosis has altered the clinical follow up and management, with the primary goal being the deceleration of disease progression. Depending on the eventual manifestation of Stargardt 1 or RP, optical aids or therapeutic interventions may substantially improve his daily life.

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P04.046.A Novel copy number variation and coding variants in Saudi patients with X-linked retinitis pigmentosa

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Background: X-linked retinitis pigmentosa (XLRP), a severe form of progressive retinal degeneration, accounts for 15% of RP cases. It is primarily caused by mutations in two genes: RPGR (OMIM #300455), responsible for most cases, and RP2 (OMIM #312600). This study investigates four unrelated XLRP Saudi families.

Material and methods: Six male patients from four Saudi families, ranging between 5–51 years, were recruited for this study. Whole exome sequencing (WES) NGS based copy number variation (CNV) analysis and was performed in the four index cases of each family followed targeted mutation screening in the affected ones.

Results: All studied patients displayed typical clinical features of profound visual loss, nyctalopia nystagmus and variable presentation of retinal dystrophy in the fundus. WES revealed hemizygous likely causative variants in all studied patients. Three distinct known variants in *RPGR* were found in five patients as follows: a nonsense mutation (c.1544T>G; p.(Leu515*) in (1/4 family; 25%), and a frameshift mutations (c.2236_2237del; p.(Glu746Argfs*23) in (2/4 family; 50%) including four affected males that might suggest a founder effect in the Saudi population. In one family we identified a hemizygous likely pathogenic by NGS CNV analysis a deletion of chromosomal region chrX:46712889-46719557, encompassing exons 2–3 of the *RP2* gene.

Conclusion: Our study broadens the genetic spectrum of RPGR-related XLRP, identifying known variants in 75% of families (five patients) and discovering a novel CNV in the RP2 gene. This emphasizes the value of NGS-based CNV analysis in uncovering missing heritability.

C.O.I. Disclosure:

B. Almoallem: None.

P04.047.B Familial steep corneas in posterior polymorphous corneal dystrophy 3 due to a novel ZEB1 gene mutation

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Background: Posterior Polymorphous Corneal Dystrophy (PPCD), particularly PPCD3 caused by ZEB1 mutations, affects the corneal endothelium and Descemet's membrane, with features including vesicular corneal opacification, potential corneal ectasia, and steep curvature in some cases.

Material and methods: Ophthalmic examinations and corneal curvatures analyses were performed for all patients. Whole exome targeted gene panel sequencing was performed for one patient. Pathogenic variant confirmation and segregation of variant was performed for all four patients using Sanger sequencing.

Results: A mother and her 3 children presented with bilateral diffuse corneal opacities, vesicular aggregates at the Descemet's membrane level and endothelial stippling, compatible with the diagnosis of posterior polymorphous corneal dystrophy. All patients had steeper than average keratometry readings with normal globe axial lengths and refractive moderate to extremely high myopia, ranging from −2 diopters to −16.5 diopters (spherical equivalent). The younger patients had more severe findings, with the youngest, an 11-months old female, presenting with keratometry readings of 66.72 × 69.48 @ 132° and 66.10 × 67.32 @ 34° in the right and left eyes, respectively. The *ZEB1*:c.794-1G>A;chr10-31809053G>A; NM_001174096.2 mutation was detected in all patients.

Conclusion: We describe a novel *ZEB1* mutation associated with posterior polymorphous corneal dystrophy, steep non-keratoconic corneas and myopia. Posterior polymorphous corneal dystrophy 3 should be considered not only as an endothelial pathology but also as an ectatic disorder and ruled out in young children presenting with high myopia.

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E. Banne: None. **T. Koval:** None. **E. Neimark:** None. **O. Spierer:** None.

P04.048.C Evaluation the effect of *COL4A1*R1656S mutation on the progressive retinopathy

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Background: Mutations in collagen type IV alpha 1 (*COL4A1*) are associated with multisystem abnormalities affecting mainly the brain and the retinal vasculature. We identified a pathogenic variant R1656S in *COL4A1* in patients with retinopathy, anterior segment dysgenesis, and cataract, and aimed to evaluate the molecular effect of R1656S-mutation in MDCKII cells.

Material and methods: MDCKII cells were transiently and stably transfected using Effectene Transfection Reagen (Qiagen) with expression vectors containing WT *COL4A1* and mutant R1656S along with GFP (pCMV-eGFP, Genecopea). ZO-1 (Tight junction protein) and GM-130 (Golgi marker) immunofluorescence, as well as TRITC-Phalloidin staining of actin cytoskeleton and cell nuclei with DAPI was performed on confluent cells.

Results: Using mammalian expression system based on transient and stably transfected MDCKII cells, we assessed the colocalization of F-actin cytoskeleton, ZO-1, GM-130, and COL4A1 proteins. No changes were found in cell morphology, ZO-1, GM130 and actin cytoskeleton structure but we observed differences in cell growth, mitotic index, and distribution of WT and R1656S-COL4A1. In cells expressing WT-COL4A1, its colocalization with F-actin filaments and ZO-1 close to the cell periphery was detected. This localization was less pronounced or absent In cells expressing R1656S-mutant. A strong signal for the *COL4A1*-R1656S was detected in close proximity to the Golgi apparatus.

Conclusion: Our findings explain the mixed phenotype of epithelial changes used as model system, which could be related to the retinal epithelial changes associated with optic nerve dysgenesis and secondary glaucoma observed in our patients, and suggest that carriers of *COL4A1*-R1656S may be at elevated risk of retinopathy.

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P04.049.D InsightRP2: a global patient registry for *RP2*-associated X-linked retinitis pigmentosa

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Background: Retinitis pigmentosa (RP) represents a genetically heterogeneous group of inherited retinal dystrophies (IRD) characterized by progressive photoreceptor degeneration leading to visual impairment. Mutations in the *RP2* gene account for approximately 20% of X-linked retinitis pigmentosa, a particularly severe and early-onset form of the disease (1,2). RP collectively qualifies as a rare disorder, which, by definition, affects less than 5 in 10,000 people. However, *RP2*-associated RP by itself is much rarer and this aspect hinders participation in rare disease studies and access to evolving treatments such as gene therapies. Patient registries serve as important tools to engage patients who may otherwise face hurdles in connecting with rare disease studies.

Material and methods: We have created a collaborative online registry database within the REDCap® framework. A clinical questionnaire, specific to *RP2* related medical issues, as well as online consent forms were created.

Results: The InsightRP2 registry is tailored to document detailed clinical and genetic data of individuals with *RP2*-associated RP. It entails a clinical questionnaire to be filled out by the participant and / or their physician, and allows for storage of medical reports, genetic reports, and imaging files for further analysis, thus facilitating natural history studies, as well as research into pathogenesis and therapy.

Conclusion: By implementing a disease specific patient registry, we aim to help linking individuals affected by *RP2*-associated RP from Germany and around the world with physicians and researchers intending to better understand the clinical manifestations and progression of the disease and eventually treat this rare condition.

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P04.050.A A novel splice variant in *COL4A1* results in retinal arterial tortuosity and HANAC syndrome in a multi-generational family

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Background: *COL4A1*-related disorders are multisystem diseases characterized by aberrant collagen IV fibers and disrupted basement membranes. We identified a novel splice-site variant (c.3876+5G>A) in *COL4A1* in a multi-generational family with intracerebral vasculopathy and retinal arterial tortuosity.

Material and methods: Ophthalmic exams, imaging and clinical genetic tests were performed for the proband, her affected father and son, and unaffected daughter. Dermal fibroblasts from the proband were harvested and used for RNA and protein analysis. RT-PCR and Sanger sequencing were performed on fibroblast-derived RNA to evaluate whether the variant in *COL4A1* disrupted normal splicing. Western blotting was used to assess protein expression using a COL4A1 antibody.

Results: Molecular testing identified a *COL4A1* splice-site variant in affected family members but not in the unaffected daughter. RNA analysis revealed skipping of exon 43 (of 52), resulting in a frameshift and premature termination codon ten amino acids downstream of the variant. Further RNA analysis using a nonsense-mediated RNA decay (NMD) inhibitor indicated that the mutant transcript is likely degraded by NMD. The absence of the predicted truncated protein on Western blotting provided further support for this hypothesis.

Conclusion: We highlight a *COL4A1*-related disorder, diagnosed by ophthalmology, in a family presenting with retinal arterial tortuosity and intracerebral vasculopathy. Functional analyses enabled upgrading the variant to likely pathogenic, solidifying the diagnosis of this rare disorder in this family and enhancing our understanding of basic genetic mechanisms underlying *COL4A1*-related disorders.

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P04.052.C Diagnosis of an overlooked rare disease: a case of Bardet-Biedl syndrome

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Background: Bardet-Biedl Syndrome (BBS) is a rare autosomal recessive ciliopathy due to primary ciliary dysfunction and is characterised by multisystemic involvement. The major features of the syndrome include retinal rod-cone dystrophy, obesity, polydactyly, intellectual disability, hypogonadism and renal anomalies. Additionally, minor findings related to various systems have been reported.

Case Presentation: A 55-year-old woman whose parents had consanguineous marriage was referred to our department from ophthalmology clinic with a pre-diagnosis of retinitis pigmentosa due to visual loss. The patient's medical history revealed that she had progressive visual loss, recurrent nephrolithiasis and haematuria since the age of 10 years. The patient's family history included visual impairments, renal problems, and obesity at various degrees. Brachydactyly, abdominal obesity, and hallux valgus were noted on physical examination. The patient was screened for additional abnormalities using imaging techniques and blood tests. Whole exome sequencing analysis revealed a homozygous c.479G>A variant in the *BBS1* gene. The patient met sufficient diagnostic criteria for BBS, received genetic counseling about the disease and referred to the appropriate departments.

Conclusion: Although the early-onset retinitis pigmentosa is one of the distinguishing features of BBS, the patient's diagnosis was delayed because the symptoms were evaluated separately and the association with the syndrome was overlooked on previous assessments. The diagnosis of BBS could have been missed if the patient had only been examined with genetic testing based solely on the referral pre-diagnosis. This case highlights the importance of multidisciplinary approach and genetic analyses in the diagnosis and management of a rare hereditary disease.

C.O.I. Disclosure:

H.S. Simsir: None. **H. Kucuk Kurtulgan:** None. **M.E. Yildirim:** None.

P04.053.D Mutations in the ciliary transport gene IFT140 cause syndromic congenital retinal dystrophy

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Early-onset, severe retinal dystrophy can be isolated or syndromic, presenting as part of an underlying systemic disease. Mainzer-Saldino syndrome, a rare systemic ciliopathy characterized by skeletal and renal disease, is caused by recessive mutations in the intraflagellar transport 140 chlamydomonas homologue (IFT140) gene. We present a series of 13 cases of early-onset retinal dysfunction with confirmed IFT140 mutations from 8 unrelated Saudi families belonging to 3 well-known tribes. All carried the same homozygous missense IFT140 mutation (c.1990G>A; p.Glu664Lys) except for a single family, which included 4 affected subjects, 3 of whom were aborted fetuses, with compound heterozygous pathogenic IFT140 variants (c.1525-1G>A and c.1990G>A; p.Glu664Lys). Severe retinal dystrophy was present in all living subjects, phenotypically apparent as hyperopia, nystagmus, nyctalopia, poor vision and nonrecordable full-field electroretinography. All affected individuals had skeletal

abnormalities, and neurological abnormalities were common, but there was no evidence of chronic renal failure.

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P04.055.B Genetic diagnosis in sensorineural hearing loss: clinical outcomes following cochlear implantation in two patients with Zimmerman-Laband syndrome (case series)

Sarah Chamieh

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Background: The gold standard recommendation for congenital sensorineural hearing loss (SNHL) care is cochlear implantation (CI). Adjusting for confounding factors such as developmental comorbidities is crucial when assessing expected outcomes of the procedure for the patients, their families, and their medical teams.

Case series: We describe two clinical cases of Zimmerman-Laband syndrome associated with two different ATP6V1B2 pathogenic variants and highlight the benefit of molecular diagnosis in a context of overlapping syndromic phenotypes.

Conclusion: Through the case study of CI in congenital syndromic SNHL, we demonstrate the importance of genotype-phenotype relationships in assessing clinical outcomes, and present genetic molecular diagnosis as a crucial tool in managing patient and family expectations.

C.O.I. Disclosure:

S. Chamieh: None.

P04.056.C Unveiling the genetic landscape of visual impairment in North Macedonia: insights from whole exome sequencing

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Background: Visual impairment encompasses a highly heterogeneous group of disorders, with inherited retinal dystrophies being the most common cause of severe, irreversible vision loss. Over 300 genes contribute to the molecular basis of visual impairment, making genetic testing essential for diagnosis, genetic counselling and prognosis.

Material and methods: Whole Exome Sequencing (WES), followed by bioinformatics analysis and ACMG classification, was performed on 44 patients referred to RCGEB for molecular diagnosis of various types of visual impairment.

Results: Causative pathogenic variants were identified in 34 (77.3%) of the patients. Among the 35 distinct pathogenic variants, nine were novel. In two patients (4.5%), variants of uncertain significance (VUS) in autosomal dominant (AD) genes (RP1L1/UNC119) were detected, while in four patients (9.1%) only a single pathogenic variant in an autosomal recessive (AR) gene was found. The *ABCA4* gene was the most frequently affected AR gene (4/34; 11.8%), followed by *FAM161A* and *PCARE* (2/34; 5.9% each). Pathogenic variants in the X-linked *RPGR* gene were identified in two male patients. Additionally, thirteen different AR genes (*ALMS1; CDHR1, CYP1B1, CNGB3, EYS, MERTK, NRL, RDH12; RP1, PDE6C, SPATA7, TSPAN12, USH2A)* and 3 AD genes (*BEST1, CRX* and *PAX6)* were causative in only one patient each.

Conclusion: The implementation of WES led to the identification of disease-causing variants in 77.3% of patients with visual impairment, enabling more accurate prognoses, optimized therapeutic approaches, and appropriate genetic counselling. Further analysis is required for patients where VUS variants or a single pathogenic variant in AR conditions were identified.

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P04.057.D A novel, recurrent, population-specific variantp.(Lys232*) in the *CACNA1F* genein Familial type 2 X-linked incomplete congenital stationary night blindness

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UMC Ljubljana, Centre for Mendelian Genomics, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia.

Background: X-linked congenital stationary night blindness (CSNB) is a nonprogressive retinal disorder characterized by decreased visual acuity and impaired night vision, with an estimated prevalence ranging from 1:5,000 to 1:25,000. We report a novel recurrent pathogenic variant in the *CACNA1F* gene, identified in two Slovenian families with a total of four affected male family members.

Material and methods: All four patients were referred for whole-exome sequencing (WES) due to impaired retinal function, suspected to be an incomplete form of X-linked CSNB. Segregation analysis was performed to determine the origin of the identified variant.

Results: A pathogenic hemizygous frameshift variant NM_005183.4:c.694A>T, p.(Lys232*), was identified in all four patients. In the first family, the variant segregated with the X-linked inherited clinical phenotype in three males. However, segregation analysis could not be performed in the second family.

Conclusion: Here, we report a novel, recurrent, population-specific pathogenic variant c.694A>T, p.(Lys232), in the CACNA1F gene, associated with X-linked congenital stationary night blindness.

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P04.058.A Disrupted *GRHL2* transcriptional activity as a mechanism of autosomal dominant hearing loss development (DFNA28)

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Background: *GRHL2* is one of over 50 genes linked to autosomal dominant hearing loss (ADHL) and is also implicated in cancers. It encodes a transcription factor. Only few ADHL-related *GRHL2* pathogenic variants have been reported and their mode of action

remains unclear. This study aimed to identify the genetic basis of ADHL in a multigeneration family with progressive hearing loss (HL) and explore the molecular mechanism of DFNA28 *GRHL2* mutations.

Material and methods: Genomic DNA from the proband and family members (n = 8) was analyzed using a 237-gene HL panel and Sanger sequencing. Expression vectors for four ADHL-related *GRHL2* variants were generated, and their transcriptional activity was assessed in HEK293T cells using a luciferase reporter assay.

Results: A novel heterozygous *GRHL2* variant (c.1061C>T; p.Ala354Val) segregating with HL was identified within the DNA-binding domain. Functional assays showed reduced transcriptional activity for this variant and two others (c.1258-1G>A, p.Gly420Glufs*111; c.1276C>T, p.Arg426*). In contrast, a variant affecting the dimerization domain (c.1609_1610insC, p.Arg537-Profs*11) strongly activated the GRHL-responsive promoter.

Conclusions: The pathogenicity of novel missense variant was supported by functional assays. *GRHL2* mutations can suppress or activate transcription depending on variant location—DNA-binding domain variant causes haploinsufficiency, while dimerization domain variant shows gain-of-function effect. This study provides new insights into *GRHL2*-related hearing loss mechanisms.

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P05 Internal organs and endocrinology (Lung, Kidney, Liver, Gastrointestinal)

P05.001.B A broader genetic testing approach in Nephropathies: towards a better diagnosis

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Background: Kidney diseases (KD) have a high incidence in Portugal. Whole exome sequencing (WES) is widely used to determine the genetic etiology of KD. However, KD's high genetic and phenotypic heterogeneity poses a challenge.

Material and methods: We present an overview of genetic testing by WES, from 2010 to 2024. Our cohort of 487 patients displays a wide range of phenotypes.

Results: Until mid-2023, genetic panels ≤85 genes were requested. 21.6% (n = 88) of the patients (n = 406) presented pathogenic (P) or likely pathogenic (LP) variants. The remaining either presented variants of uncertain significance (VUS) or a single pathogenic variant in a gene with an autosomal recessive inheritance (n = 148), or rendered a negative result (n = 170). All reported VUS were reinterpreted and 21 presented a different classification: 6 patients with a VUS received an upgrade in classification (LP), while 15 were downgraded to benign. In 2023, besides Copy Number Variation (CNV) analysis, we implemented broader panels. Since then, 29.6% (n = 24) of the 81 index cases, in which a broader first analysis was requested, had LP/P variants. We detected a deletion and a microduplication, which would fail to be reported without CNV analysis. Moreover, reanalysis of 24 negative samples employing broader genetic panels rendered a positive result in four of them (17%).

Conclusion: Our findings emphasize the significance of reevaluating variant classification and using a larger number of genes in nephropathy genetic testing. Effective treatment of the patients, as well as adequate screening and family counseling, are made possible by an accurate initial diagnosis.

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P05.002.C A novel laterality disorder with ciliary dysfunction caused by *PIH1D2* loss-of-function

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Background: In normal human anatomy, the position and orientation of thoraco-abdominal organs and major blood vessels exhibit a distinct asymmetry along the body's left-right axis. Disruption of the left-right patterning process during embryogenesis gives rise to laterality disorders, characterized by abnormal arrangement of internal organs and great vessels, often combined with congenital malformations. We aimed to characterize the genetic defect in two infants with left isomerism heterotaxy, caused by bi-allelic loss-of-function variants in a novel disease-causing gene.

Methods: Trio genome sequencing was performed for each family. Primary dermal fibroblasts from *Patient 1* were generated and used for RNA extraction to study the detected splice variant in *PIH1D2*, for immunofluorescence staining and for in vitro ciliogenesis assays.

Results: We characterize a novel laterality syndrome of disrupted left-right asymmetry, including dextrocardia, polysplenia and biliatry atresia. Genome sequencing revealed bi-allelic loss-of-function variants in the *PIH1D2* gene in each patient. Immunofluorescence staining confirmed protein loss in *Patient 1* fibroblasts. A ciliary defect was supported by a low nasal nitric oxide level in *Patient 2*, and by a significant reduction in ciliogenesis of *Patient 1* fibroblasts upon serum starvation ($p = 0.0004$).

Conclusion: This study provides evidence of a novel left isomerism heterotaxy syndrome caused by bi-allelic loss-of-function variants in *PIH1D2*. Our data is supported by previous studies in mutant zebrafish exhibiting heart-looping defects, and other primary ciliary dyskinesia syndromes caused by genes belonging to the PIH protein family. Additional patients will provide better characterization of the full phenotypic spectrum and natural history of this disorder.

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P05.003.D Functional assessment of *NPHS2* pathogenicity - the world of interallelic interactions

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Background: *NPHS2*, encoding podocin, is the most frequently mutated gene in podocytopathies. The pathogenicity of its most frequent R229Q variant depends on the trans-associated variant, which makes the pathogenicity assessment of unknown R229Q-associations particularly challenging. We previously showed that podocin shortens the distance between neighboring nephrin molecules, and here aimed to determine its potential in the pathogenicity assessment of unknown associations.

Material and methods: HEK293 cells were transfected with nephrin constructs labelled extracellularly with YPet or mCherry encoded in pEGFP-N1 plasmids, and a pKK-BI16 plasmid expressing two different podocin variant. FRET efficiency was measured between YPet and mCherry in living cells, 48h after transfection. Measurements were repeated nine times in three experiments.

Results: While all benign podocin associations (R229Q-wt, R229Q-R138Q, R229Q-R229Q, R229Q-V290M) increased the FRET efficiency between nephrin molecules ($p < 0.005$), none of the pathogenic associations (R229Q-A248V, R229Q-A297V, R229Q-F344Lfs, R229Q-R291W) exerted such an effect. The difference between benign and pathogenic variants/associations was highly significant ($p = 1.19 \times 10^{-33}$), and a cut-off value could distinguish them in 100% of the measurements.

Conclusion: Benign R229Q podocin oligomers significantly shorten the distance between nephrin molecules in cis, while pathogenic oligomers are unable to do so. This mechanism explains the molecular basis of the interallelic interactions, and is also specific enough to help the distinction of pathogenic and benign R229Q associations.

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P05.004.A Genome-wide aggregated *trans*- effects analysis implicates deficient Type III interferon signalling as a key cause of inflammatory bowel disease

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Background: The "omnigenic" sparse effector hypothesis postulates that the polygenic effects of common SNPs on a typical complex trait are mediated by trans- effects that coalesce on expression of core genes. The objective of this study was to identify core genes and core pathways for inflammatory bowel disease.

Material and methods: We tested for association of inflammatory bowel disease with genome-wide aggregated trans-effects (GATE) scores for gene expression in 7907 cases and 451198 non-cases in UK Biobank.

Results: Inflammatory bowel disease was inversely associated with predicted expression of six interferon-stimulated genes - I*FIT1, IFI44, HERC5, MX1, IFI44L, RSAD2* - that were regulated by the same trans-eQTLs, and with predicted levels of the Type III interferon IFN-λ1 encoded by *IFNL1*. Ten other genes were identified as possible core genes based on GATE score associations with inflammatory bowel disease, of which three were supported by a reported GWAS hit within 200 kb of the transcription site, and three - *CCDC50, VCAM1, HAVCR2* - by effects on colitis in a mouse model. Of these ten genes, three have roles in the innate immune system, one in the adaptive immune system, and one in leucocyte trafficking.

Conclusion: These results establish a key role of deficient Type III interferon signalling in the aetiology of inflammatory bowel disease, supported by other evidence including previously reported GWAS associations, monogenic disease caused by rare variants, clinical studies of gene expression and experimental perturbation in a mouse model. More generally, these results identify possible therapeutic targets.

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P05.005.B Early diagnosis of Wolfram syndrome type 1: insights into diabetes insipidus presentation and genotype-phenotype correlation

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Background: Wolfram syndrome type 1 (WS1) is a neurodegenerative disorder caused by biallelic WFS1 mutations, primarily characterized by the association of diabetes mellitus (DM), optic atrophy (OA), diabetes insipidus (DI), and hearing loss (HL). Early DI diagnosis in WS1 may be missed as its polyuria and polydipsia symptoms overlap with those of DM. This study used a structured questionnaire to detect DI and examined genotype-phenotype correlations in a Turkish Type 1 DM cohort.

Material and methods: We retrospectively analyzed 1,278 pediatric patients with Type 1 DM. A polyuria/polydipsia questionnaire was administered during follow-ups to identify DI symptoms masked by DM. Eighteen patients with positive results underwent WFS1 genetic testing. The identified variants were assessed by ACMG criteria.

Results: Six patients (33%) were diagnosed with WS1, carrying seven different *WFS1* variants: four missense mutations, one in-frame duplication, and two frameshift variants, including two novel variants (c.2104G>T and c.1727_1744dup). The median age at diagnosis of WS1 was 9 years 1 month. Three patients had OA, and one had HL, reflecting phenotypic variability. Two siblings with the same frameshift mutation showed different ages of onset and different phenotypic presentations, suggesting intra-familial variability.

Conclusion: DI may precede OA in WS1, and early detection by a simple questionnaire could enable early diagnosis. The present study emphasizes the importance of genetic screening in patients with Type 1 DM presenting with symptoms of DI and highlights the need for functional studies and longitudinal follow-up to

better define genotype-phenotype correlations in order to improve clinical management.

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P05.006.C Genetic and clinical characteristics of familial pulmonary fibrosis patients and relatives

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Background: In the majority of familial pulmonary fibrosis (FPF), defined as the presence of fibrotic interstitial lung disease in at least two first- or second-degree relatives, the underlying genetic cause remains unknown, complicating the screening approach for relatives.

Material and methods: We included 132 families (155 patients and 213 relatives) and performed in-depth genetic and clinical characterization of this cohort. Analysis of the pulmonary fibrosis gene panel, genotyping of the *MUC5B* promoter polymorphism (rs35705950) and telomere length (TL) assessment (using flow-FISH) was done in patients. For families with a negative gene panel result, the whole exome data were analyzed for variants in candidate genes. Relatives took part in a multimodal screening program (pulmonary function test and high-resolution CT-scan, besides genetic testing).

Results: A (likely) pathogenic (LP/P) variant was detected in 23/132 (17,4%) families, mostly in the telomere-related genes (*RTEL1*, *TERT*, *PARN*, *TERC*). In 2 families we found a LP/P variant in a surfactant-related gene (*SFTPC*, *SFTPA2*). In 28/132 (21.2%) families a variant of unknown significance (VUS) was detected. The c.308T>C p.(Leu103Pro) VUS in the *TERT* gene (NM_198253.2) was seen in 3 independent families and might be a founder variant, which we will verify by haplotype analysis. Furthermore, we detected rare variants in the *KIF15* candidate gene segregating with the phenotype in 5 families. A common *KIF15* variant, thought to be a risk allele, was overrepresented in our cohort. About 12% of relatives had interstitial lung abnormalities/disease on screening.

Conclusion: In-depth genetic characterization will facilitate follow-up and early detection in relatives.

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P05.007.D Burden of potential kidney failure in apparently asymptomatic patients

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Background: Chronic Kidney Disease (CKD) is a major health concern due to its high morbidity and asymptomatic nature in early stages. Early detection is essential for timely intervention, reducing complications and mortality. Despite its prevalence, only a small percentage of affected individuals are aware of their condition. Genetic screening offers a promising approach for identifying at-risk individuals before symptoms arise.

Material and methods: This study analyzed 8,376 exomes from patients evaluated for conditions unrelated to kidney disease. To determine the prevalence of genetic predisposition among asymptomatic individuals, we searched for pathogenic and likely pathogenic (P/LP) variants in 67 CKD-associated genes.

Results: P/LP variants were identified in 6.4% of individuals across 60 genes associated with CKD. Among the most notable findings, variants in the Collagen type IV genes were present in 1.4% of cases, underscoring their significant role in CKD pathogenesis. Additionally, variants in autosomal dominant *ACTN4, TRPC6, INF2, LMX1B, WT1, APOL1, PAX2, ANLN, FN1* and *MYH9* accounted for another 0.84%. These findings suggest a significant genetic contribution to CKD development and highlight many individuals carrying genetic risks without clinical manifestation.

Conclusion: The genetic predisposition to CKD in asymptomatic individuals is underestimated. Integrating genetic screening into clinical practice can enhance early diagnosis, allowing for personalized treatment strategies and improved patient outcomes. Future research should focus on optimizing screening protocols and evaluating the long-term benefits of genetic risk assessment in CKD management. Genetic characterization may become a key tool in preventing disease progression and advancing precision medicine in nephrology.

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P05.008.A Investigation of the molecular mechanism underlying IBD phenotypes through a large meta-analysis of scRNAseq data

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Background: Inflammatory Bowel Disease (IBD) is one of the most prevalent gastrointestinal diseases worldwide. However, despite the advent of new therapeutic agents, patients deal with debilitating symptoms and a high non-response rate. Studies such as Martin et al. (Cell 2019) and Smillie et al. (Cell 2019) have provided insights into the disease mechanism, yet their limited statistical power due to small cohort sizes impedes a comprehensive understanding. To overcome these limitations, we propose a large-scale meta-analysis by combining and harmonizing independent IBD scRNAseq and genotype studies.

Material and methods: We developed a standardized pipeline on gut mucosa scRNAseq and genotype data to align quality control, preprocessing, cell type annotation, differential proportion analysis, differential expression, and eQTL mapping.

Results: We harmonized and integrated two Chron's Disease (CD) and two Ulcerative Colitis (UC) scRNAseq datasets. We obtained extensive datasets comprising over 485,000 cells from 150+ individuals for CD and over 290,000 cells from 30+ individuals for UC. Moreover, we performed cell abundance

analysis of inflamed vs non-inflamed samples in both UC and CD datasets. The analysis shows a concordant behavior between UC and CD upon inflammation in 67% of cell types, while some disconcordant cells include CD8 T cells, immature enterocytes, and glial cells.

Conclusion: We created a standardized pipeline for harmonizing scRNAseq and genotype data applied to four IBD datasets. Our preliminary analysis shows the presence of some relevant differences among the diseases. Further investigation will deepen our understanding of IBD pathomechanisms, aiming to improve therapeutic strategies for patients.

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P05.009.B Rare renal disease focal segmental glomerulosclerosis (FSGS) - correlation of genotype and clinical and histopathological phenotype in adult Central European patient cohort

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Background: Focal segmental glomerularsclerosis (FSGS) is a clinically and genetically heterogeneous glomerular disease that manifests itself by proteinuria. About 15% of FSGS patients progress to renal failure. A portion of the FSGS cases is of a monogenic etiology. The prevalence of adult-onset genetic FSGS is considered underestimated and its clinical, genetic and histological features have not been clearly described.

Material and methods: Multicentric study, cohort: FSGS adult patients steroids resistant, kidney biopsy confirmed.

Methods: whole exome sequencing, whole genome sequencing, bioinformatics, clinical data analysis, re-analysis of kidney biopsies Columbia classification.

Results: The analysis of 225 families cohort yielded 16% (n = 35) index patients with genetic FSGS, out of these, 50% probands carried causal variant in Alport syndrome phenocopy genes *COL4A3,4,5* and 50% in various podocyte-specific genes. We divided the patients according to genotype into 3 groups: with podocyte variants (P), Alport variants (A) and without genetic variant (N) and correlated them with the clinical and histologic phenotype. We established statistically significant differences among the groups in manifestation age (P 23y/A 37y/N 39y), edemas presence (P 18%/A 0%/N 60%) and frequency of kidney failure (P 45%/A 29,4%/N 15%). Kidney biopsies re-analysis using Columbia Classification showed the majority of NOS lesions and highest fibrosis level (42,9% level 2) in P group. Tip lesion prevailed in N group (61,1%).

Conclusion: This is the first large-scale adult-onset genetic FSGS study in Czech population. It brings new insights into genetic and clinical features of the FSGS in adults and the results will improve personalized medical care.

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P05.010.C Epidemiology of monogenic diabetes in the Czech Republic

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Background: Monogenic diabetes results from a single-gene defect affecting pancreatic beta cells. Accurate diagnosis enables personalized treatment. While precise European prevalence data are lacking, the U.K., a global leader in monogenic diabetes research, reports an estimated prevalence of 24.8/100,000 and an observed prevalence of 5.8/100,000. This study assessed diagnostic trends and determined the minimum prevalence of monogenic diabetes in the Czech Republic.

Material and methods: Clinical and genetic data were collected from individuals suspected of having monogenic diabetes referred for genetic testing and registered in the National Registry for Monogenic Diabetes between 1999 and 2023. Covariates were analysed using the t-test, and categorical variables with $\chi 2$.

Results: By 2023, we registered 1,879 families (i.e., probands) with suspected monogenic diabetes, and 728 (39 %) received a confirmed diagnoses (1,473 individuals from the Registry in total). The causative variant was identified in 13 different genes, most commonly in *GCK* (69 %), *HNF1A* (15 %), and *HNF4A* (8 %). The minimum prevalence of monogenic diabetes was 13.6/100,000. The number of referred probands increased from 42 between 1999–2001 to 142 in 2023. The detection rate has stabilized since

2011 at 30–40 %. The average age of diabetes diagnosis was 15.5 (± 9.4) years, with 76 % of probands having a family history of diabetes.

Conclusion: We reported an increasing referral rate and stable detection rate of monogenic diabetes in the Czech Republic. However, considering the U.K. prevalence estimates, about half of the Czech patients with monogenic diabetes remain un- or misdiagnosed.

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P05.011.D The role of the ciliary TRIM8 protein in focal segmental glomerulosclerosis and neurodevelopmental syndrome

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Background: TRIM8 is an E3 ubiquitin ligase involved in cell cycle progression, that interacts with master regulators of mitotic spindle assembly. Our recent transcriptomics and proteomics studies demonstrated that TRIM8 localizes at centrioles and regulates centrosomal protein CEP170. Also, it is a novel ciliary protein located at the basal body, and TRIM8 depletion causes a significant reduction in primary cilia number and length. Notably, heterozygous dominant variants on *TRIM8* cause a neuro-renal syndrome (FSGSNEDS), characterized by developmental delay and podocytopathy.

Material and methods: We engineered human podocytes using CRISPR/Cas9 mimicking patients' variants, and then fully characterized them morphologically and functionally, studying cellular permeability, endocytosis, viability and apoptosis. Then, we performed RNASeq to highlight deregulated genes and pathways associated with TRIM8 variants.

Results: Heterozygous TRIM8 podocytes displayed lower viability and increased apoptosis compared to their isogenic controls. Also, they showed altered cytoskeletal network, increased prolapse between cell-cell junctions with higher endosomal escape. Interestingly, our transcriptomics analysis confirmed among the most deregulated pathways those involved in cell adhesion, with a significant down-regulation of several cadherins, along with catenin complex, inflammation and synaptic organization.

Conclusion: Although preliminary, our data present a new role for TRIM8 in cell adhesion and cytoskeletal integrity, that we are deepening to understand FSGSNEDS patients' podocytes dysfunction. To this aim, we are generating patients' hiPSCs-derived kidney organoids to analyze them by scRNAseq and proteomics.

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P05.012.A Pathogenic mechanisms in Hirschsprung's Disease: insights from transcriptomic analysis

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Background: Hirschsprung's disease (HSCR) is a congenital disorder arising from an impaired migration and differentiation of neural crest cells (NCCs), resulting in the absence of enteric neurons in the distal colon. While mutations in the *RET* proto-oncogene are recognised as contributing factors, the potential role of extracellular matrix (ECM) remodeling and transcriptional regulation remain poorly understood.

Material and methods: Intestinal tissues from ganglionic (HG) and aganglionic (HA) regions were collected from 13 male patients with short-segment HSCR (S-HSCR). RNA sequencing identified differentially expressed genes (DEGs) (fold-change >1.5, FDR <0.05), and pathway enrichment analysis was performed to explore molecular mechanisms underlying HSCR pathogenesis.

Results: Results suggest disruptions in ECM components (*FERMT1, MMP2*, and *ITGA6)*, which may impair NCC adhesion and migration, thus contributing to the absence of enteric neurons in the distal colon. In addition, downregulation of the transcription factors *SOX9* and *HOXB5*, key regulators of RET signalling and NCC differentiation, was also observed. Furthermore, the study identified deficiencies in *FGFR4* expression and MAPK/ERK signaling, which may further impair pathways critical for the development of the enteric nervous system (ENS). The analysis also revealed alterations in Wnt signaling, axon guidance, and inflammatory responses, highlighting their roles in HSCR.

Conclusion: This study proposes that ECM remodeling, transcriptional dysregulation, and signaling pathway impairments collectively contribute to HSCR. These findings reveal therapeutic targets, such as restoring ECM integrity or enhancing RET signaling. While further validation is required, this work provides a suitable framework to explore novel interventions and advancing our understanding of HSCR pathophysiology

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P05.013.B Reverse phenotyping and genetic stratification in Cystic Kidney Disease: new perspectives for diagnosis and personalised therapy

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Background: Cystic kidney disease (CKD) comprises a heterogeneous group of disorders characterised by the formation of renal cysts and progressive deterioration of kidney function. While variants in tubular genes such as *PKD1* and *PKD2* are well

established in autosomal dominant and recessive polycystic kidney disease (ADPKD and ARPKD), emerging evidence suggests that additional genes involved in renal morphogenesis may contribute to disease pathogenesis. This study investigates the role of reverse phenotyping in the genetic stratification of CKD patients, emphasising how genotypic characterisation can refine differential diagnosis, prognosis, and therapeutic decision-making.

Methods and results: Variants in *PKD1* were identified in 59.3% of cases (seven males and nine females). Additionally, pathogenetic variants were detected in collagen type IV genes (*COL4A3*, *COL4A4*, *COL4A5*) in 18.5% of cases, *ALG8* in 7.4%, and *HNF1B* in 3.7%. One female harboured two biallelic variants in *PKHD1*. Among individuals with *PKD1* mutations, variants were also found in *CFHR3* and *GANAB* genes. In three cases, no mutations were identified despite a clear polycystic phenotype.

Conclusion: Reverse phenotyping mitigates the limitations of phenotype-based diagnosis alone in CKD, facilitating improved prognostic stratification and personalised treatment approaches. The identification of novel genes implicated in CKD pathogenesis may pave the way for targeted therapies and optimised clinical management of affected individuals.

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P05.014.C Comprehensive diagnostic approach to identifying pathogenic variants in *SLC34A1* and *SLC34A3* genes in patients with suspected NPT2 damage

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Background: Phosphate metabolism is primarily regulated by phosphate reabsorption via NPT2a (*SLC34A1*) and NPT2c (*SLC34A3*) transporters. Pathogenic variants in these genes are associated with recessive hypercalcemia, infantile 2 (IHC2), dominant nephrolithiasis/osteoporosis, type 1 (NOH1), and hereditary hypophosphatemic rickets with hypercalciuria (HHRH). The existence of a dominant phenotype for *SLC34A3* currently remains under question.

Material and methods: 55 unrelated patients with suspected NPT2 damage underwent molecular genetic testing (WGS or WES). NGS data for 36 patients were reanalyzed, followed by functional analysis or WGS.

Results: Initial NGS analysis identified biallelic variants in 29% (7/24) of IHC2 patients and 35% (11/31) of HHRH patients. Further reanalysis, functional studies, allelic imbalance investigation, or WGS were performed for undiagnosed cases. Reanalysis detected a second variant in 7 IHC2 and 5 HHRH patients, including a recurrent pathogenic *SLC34A1* variant initially filtered due to high frequency and a synonymous *SLC34A1* variant in 3 patients, prompting a minigene assay. In HHRH, a complex allele and a synonymous *SLC34A3* variant were identified, with RNA analysis on fibroblasts clarifying pathogenicity. WGS was performed for 9 IHC2 and 15 HHRH patients, revealing 6 monoallelic *SLC34A1* cases reclassified as dominant phenotype NOH1. In 5 patients, we found missed intronic variant in *SLC34A3* confirming HHRH, 10 remained monoallelic, requiring further investigation.

Conclusion: A comprehensive diagnostic strategy with NGS data reanalysis and functional studies is crucial for accurate clinical diagnosis and can substantially enhance genetic diagnostic outcomes.

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P05.015.D Activation of hypoxia signalling favours somatic mutation in normal kidney cells

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Background: Somatic genetic alterations are progressively engraved in normal tissues with age and known to drive cancer initiation, but the molecular mechanisms determining somatic mutation accumulation are not fully elucidated. In the kidneys, 90% of clear cell renal cell carcinomas (ccRCCs) harbour mutations that inactivate the negative regulator of adaptation to low oxygen (hypoxia), i.e. the product of the *Von Hippel Lindau (VHL)* gene.

Material and methods: To assess a possible causal relationship between hypoxia signalling activation and mutation accumulation, we investigated normal kidney cells from VHL-disease patients (*VHL+/−) and* controls *(VHL+/+)*. The pattern of somatic mutations acquired in vivo was analysed by WGS of clonally amplified cells, while the in vitro pattern upon hypoxia exposure was analysed by duplex sequencing.

Results: We found in vivo accumulation of COSMIC signatures SBS1, SBS5 and SBS8 in all kidney cells. However, a subset of *VHL +/−* cells also bore the marks of SBS40b, a signature specifically found in ccRCC. These normal kidney cells showed molecular features of adaptation to hypoxia, including nuclear translocation of the HIF (Hypoxia Inducible Factor) 1 alpha transcription factor, usually repressed by VHL, and upregulation of HIF target genes. Similarly, we discovered that when *VHL+/+* normal kidney cells were subjected to in vitro hypoxia, HIF1A underwent nuclear translocation and the SBS40b signature accumulated.

Conclusion: Through the analysis of somatic mutations acquired in vivo and in vitro, we provide evidence that accumulation of the ccRCC-specific, mutational signature SBS40b can be triggered by activation of hypoxia signalling in normal kidney cells.

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P05.017.B Biallelic canonical splice site variant in *FMNL2* underlies an autosomal recessive form of renal tubulopathy

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Background: Renal tubulopathies (RTs) are a rare and heterogeneous group of genetic diseases that often result in electrolyte imbalances, metabolic acidosis, and impaired growth. Despite significant advances in molecular diagnosis, many RTs remain genetically undiagnosed. *FMNL2* gene encoding Formin-like 2 protein is known to be highly expressed in kidney tissues and is associated with mitochondrial proteins. However, its role in renal and mitochondrial pathology remains largely unexplored.

Material and methods: A 3-year-old male with renal tubular acidosis (RTA) and developmental delay was evaluated clinically and genetically (Exome sequencing). Minigene assay was performed in patient-derived fibroblasts whereas mitochondrial respiratory chain complex assay (RCC) and TEM were performed on muscle tissue to evaluate the effect of disease-causing variation in mitochondrial function and structure.

Results: The proband presented with polyuria, polydipsia, failure to thrive, and facial dysmorphism since the age of 1 year. He had elevated serum lactate levels (3.8 mmol/L), and the biochemical profile suggested distal RTA. Exome sequencing analysis revealed a canonical splice-site variant in *FMNL2*:c.783-1G>A (NM_052905.4) in homozygous state. The splice variant resulted in the deletion of exon 9, producing an aberrant transcript and likely leading to a truncated protein. RCC assay showed normal enzyme activity for C-I to C-IV, whereas ultrastructural analysis revealed abnormal mitochondria with loss of cristae.

Conclusion: Our study provides the first evidence of *FMNL2* as a novel candidate gene for renal tubulopathy and highlights its putative role in mitochondrial structure. Further, functional analysis is needed to decipher the pathological mechanism of *FMNL2*-related renal tubulopathy.

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P05.018.C Re-analysis of NGS data for Copy Number Variations (CNVs) detection greatly increases the diagnostic yield in patients with growth disorders

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Background: Growth defects frequently originate from genetic factors, involving variations in genes that influence GH secretion, skeletal development, or cellular metabolism.

Materials and methods: Using a 107-gene NGS panel targeting growth disorder-related genes, we analyzed a cohort of 362 patients (212 males and 150 females) with idiopathic and syndromic short stature. To improve the diagnostic yield, we retrospectively reanalyzed NGS FASTQ data from a subset of 65 patients, tested negative for point mutations, using enGenome eVai software (v3.5) to detect copy number variations (CNVs).

Results: Pathogenic/Likely Pathogenic (P/LP) variants were identified in 61 patients (16.8%), with *ACAN, COMP, FGFR1, FGFR2, FGFR3, GNAS, GNRHR, LZTR1, NF1, NPR2, PTPN11, SLC26A2* and SOS1 being the most frequently mutated genes. The detection rate was similar in males and females (16.5% and 17.3%, respectively). Additionally, we identified 221 Variants of Uncertain Significance (VUS) in 81 genes, whose roles require further investigation. The CNV bioinformatics analysis identified clinically relevant alterations in 9 patients, suggesting that the diagnostic rate could be increased by 13.8%. Specifically, four patients carried whole-gene deletions (*GH, NF1, GLI2, LHX3*), while three had intragenic deletions: one affecting 9 exons of *GLI3*, another 7 exons of *PHIP* and the last 1 exon of *KRAS*. Two patients exhibited intragenic duplications: one involving 2 exons of *ANOS1* and the other 7 exons of *FGFR3*. These CNVs were confirmed by MLPA.

Conclusion: These findings emphasize the added value of CNV detection in the molecular screening of growth disorders, uncovering additional pathogenic variants that are undetectable by standard variant calling.

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P05.019.D A Sibling case *with AGR2* variant causing severe respiratory and digestive symptoms

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Background: Anterior gradient 2 (AGR2) is a protein disulfide isomerase that is important for protein processing in the endoplasmic reticulum and is essential for mucin production in the digestive and respiratory tracts. Bi-allelic *AGR2* variants are known to cause recurrent respiratory infections and failure to thrive with or without diarrhea (RIFTD; MIM # 620233), although the mechanisms behind this condition remain unclear. To date, at least six pathogenic *AGR2* variants have been reported.

Material and methods: We analyzed two affected siblings in a consanguineous family who had recurrent respiratory infections and digestive symptoms, one of whom needed lung transplantation. We performed exome to identify the genetic cause of their symptoms and functional studies to investigate the effect of this variant.

Result: We identified a novel homozygous missense variant in *AGR2* (NM_006408.4, c.250A>C, p.(Ser84Arg)) in both affected siblings. Both parents had the identical variant in a heterozygous state. This variant is quite rare in the general population and is clinically compatible with RIFTD, substituting a highly conserved CXXS motif with CXXR. Through transient overexpression, Ser84Arg AGR2 decreased protein stability, and promoted aberrant dimerization under non-reducing conditions. AGR2 functions in a monomer-dimer equilibrium. Size-exclusion chromatography showed that the Ser84Arg mutant had a larger molecular size than the wild-type protein under non-reducing, but not reducing conditions, indicating that Ser84Arg enhanced intermolecular disulfide bonds.

Conclusion: We identified a novel pathogenic *AGR2* variant and indicated its abnormal monomer-dimer equilibrium as a possible mechanism involved in the pathogenesis of RIFTD.

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P05.020.A Whole-exome sequencing in kidney transplant patients: implications for chronic kidneydiseaseandprecision medicine in transplantation

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Background: Chronic kidney disease (CKD) is influenced by genetic factors that affect disease progression and transplant outcomes. This study investigates genetic variants associated with CKD in kidney transplant recipients and donors using whole-exome sequencing (WES).

Material and methods: Kidney transplant donors and recipients were recruited between 2023 and 2024 from the "University Medical Center" CF and National Center for Oncology and Transplantology (Astana, Kazakhstan). The study included 14 pediatric recipients, 55 adult recipients, and 67 kidney transplant donors, all of whom underwent WES on Illumina NovaSeq 6000.

Results: Genetic variants associated with CKD were identified in 16 adult recipients (29%), including MUC1 (4 cases, with insertions and deletions in non-canonical transcripts), COL4A3 (3 cases), and rare variants in UMOD, TBC1D8B, PAX2, ANLN, PIGA, ROBO2, NOTCH2, and ACTN4. One individual had compound heterozygous variants in LAMA5. Among pediatric recipients, 4 (29%) had significant variants in SLC3A1, MUC1, COL4A3, and GREB1L. In the donor cohort, pathogenic and likely pathogenic variants associated with autosomal dominant kidney diseases were identified in 10 donors (15%), affecting genes such as COL4A3 (2 cases), UMOD (2 cases), HNF4A, WT1, CFHR5, SEC63, COL4A5, and SLC3A1. The high frequency of the APOE:c.388T>C variant, associated with lipoprotein glomerulopathy, observed among both recipients and donors (20%), necessitates further clinical evaluation.

Conclusion: WES is a valuable tool for identifying genetic causes of CKD, providing insights for family counseling and assessing risks of kidney disease development in recipients and donors, thus underscoring the importance of genetic screening in transplant medicine.

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P05.021.B Phenotype-genotype comparison of three Czech families with Heřmanský-Pudlák syndrome

Jakub Trizuljak[1,2,3], *Zuzana Vrzalová*[1,3], *Ivona Blaháková*[1,3], *Paulína Likavcová*[2], *Kateřina Staňo Kozubík*[2,3], *Jiří Štika*[3], *Martina Doubková*[4], *Šárka Pospíšilová*[1,2,3], *Michael Doubek*[1,2,3]

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Background: Heřmanský-Pudlák syndrome (HPS) is an autosomal recessive disorder associated with oculocutaneous albinism, bleeding diathesis, granulomatous colitis, and highly penetrant pulmonary fibrosis in some subtypes. Homozygous or compound heterozygous pathological variants in AP3B1, AP3D1, BLOC1S3, BLOC1S5, BLOC1S6, DTNBP1, HPS1, HPS3, HPS4, HPS5, and HPS6 genes lead to clinical manifestation of the disease. We present a phenotypic series of four patients from three distinct families in the Czech Republic.

Material and methods: We performed whole exome sequencing (WES) in affected patients. The detected variants were confirmed by Sanger sequencing.

Results: We detected causal pathogenic variants in all three families. The proband in family one carried compound heterozygous *HPS1* variants p.(Gln503*); p.(Gln397Serfs*2); two sisters in the second family carried homozygous p.(Gln397Serfs*2) *HPS1* variant, while the proband from the third family carried homozygous p.(Arg217*) *HPS4* variant. All four patients were affected by oculocutaneous albinism and functional platelet defect. At the time of investigation, 3 out of 4 patients (75%) developed pulmonary fibrosis, with the median of onset being 50 years. None of the patients developed cytopenias, granulomatous colitis, chronic kidney failure or cardiomyopathy.

Conclusion: We present the largest phenotypic series of patients with Heřmanský-Pudlák syndrome from the Czech Republic. Early detection of HPS is essential for correct management of pulmonary fibrosis.

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P05.022.C Gitelman and Bartter syndromes: insights from a longitudinal cohort study on salt-wasting tubulopathies

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Background: Inherited kidney tubulopathies are genetic disorders affecting tubular transporter proteins, disrupting homeostasis and

causing renal and extrarenal symptoms.Gitelman syndrome (GS) and Bartter syndrome (BS) are monogenic diseases with overlapping clinical features, including hypokalemia, hypochloremic metabolic alkalosis, hyperreninism, and secondary hyperaldosteronism due to renin-angiotensin-aldosterone system activation. Given their clinical similarities, genetic studies are essential for differential diagnosis.Despite their significance, GS and BS remain poorly understood, with limited population studies on prognosis and patient follow-up.

Methods: The cohort of patients with suspected GS and BS diagnoses underwent analysis using NGS techniques. Integrated DNA Technologies (IDT, Illumina®) platforms were employed until a definitive diagnosis was reached, confirming identified variants via Sanger sequencing. Clinical assessment of this cohort occurred at the time of diagnosis, and ongoing follow-up evaluations are being conducted to monitor disease progression, particularly focusing on treatment requirements.

Results: Preliminary studies identified 39 individuals diagnosed with GS, 9 diagnosed with BS, and 5 carrying mutations in both disease-related genes. Additionally, some heterozygous GS mutation carriers exhibited clinical symptoms. All individuals underwent genetic analysis. At the time of diagnosis, potassium levels emerged as the most affected clinical parameter, with a median of 3.1 Eq/l.Significant phenotypic variability was observed, ranging from mild to severe cases, suggesting potential differences in clinical outcomes based on treatment regimens.

Conclusion: While GS is often considered asymptomatic and BS more severe, both syndromes are frequently misdiagnosed. This highlights the need for accurate genetic diagnosis, careful prognosis assessment, and continuous patient monitoring to optimize disease management and treatment strategies.

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P05.023.D Recurrent genetic variants and molecular diagnostic strategies for hereditary nephropathies in the Moroccan population

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Background: Hereditary nephropathies are a major cause of end-stage renal disease in Morocco, requiring dialysis or transplantation in ~50% of children and >15% of adults. These disorders, inherited in autosomal dominant, recessive, or X-linked patterns, present diagnostic and management challenges. Recurrent mutations play a crucial role in disease understanding and are particularly prevalent in populations with high consanguinity, such as Morocco.

Material and methods: A strategic diagnostic approach was developed, prioritizing recurrent mutation screening through targeted Sanger sequencing or multiplex PCR before utilizing nephropathy-specific gene panels via Next-Generation Sequencing (NGS).

Results: Based on the experience of the Department of Medical Genetics at the National Institute of Hygiene, in collaboration with nephrologists and pediatric nephrologists from different regions of the Kingdom, a diagnostic strategy was established for key nephropathies:

- **Primary Hyperoxaluria Type 1:** *AGXT* c.731T>C (p.Ile244Thr)
- **Infantile Cystinosis:** *CTNS* c.922G>A (p.Gly308Arg)
- **Distal Renal Tubular Acidosis Type 1b:** *ATP6V1B1* c.1155dupC
- **Hereditary Nephropathies:** *TTC21B* c.626C>T
- **Nephronophthisis:** Large deletions in *NPHP1*

Conclusion: Identifying recurrent variants is essential for improving diagnosis, personalizing care, and advancing research. Artificial intelligence (AI) integration in molecular diagnostics offers a promising approach to analyzing large genomic datasets for mutation detection. Developing innovative strategies and incorporating new technologies will help overcome challenges posed by these complex diseases

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P05.024.A Genomic and metabolomic signatures of metabolic dysfunction-associated steatotic liver disease (MASLD) subjects

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Background: Metabolic dysfunction-associated steatotic liver disease(MASLD, formerly known as NAFLD) is the leading cause of liver diseases, ranging from simple steatosis to steatohepatitis. Environmental and genetic factors influence the susceptibility to MASLD. Several non-invasive methods aim at diagnosing and predicting significant/advanced fibrosis, including untargeted metabolomics and genomics approaches. NAFLD-susceptibility is highly associated with four genetic variants:rs738409, rs58542926, rs641738, rs1260326. We aimed to identify metabolomics signatures according to the severity of the disease combining metabolomics and genetics analyses data.

Material and methods: 106 MASLD patients attending our Hepatology Unit department were genotyped by TaqMan5′-nuclease assays. We categorized patients in High-Risk and Low-Risk groups by a weighted polygenic risk score. As well, we divided patients into three severity levels to evaluate liver disease progression. Untargeted metabolomics analysis was performed through Gas Chromatography-Mass Spectrometry (Shimadzu2010SE), after metabolome extraction, purification and derivatization using MetaboPrep GC-kit.

Results: For disease severity analysis, Partial Least Squares Discriminant Analysis (PLS-DA) demonstrated progressive separation, with key metabolites including urea, lactic, pyroglutamic, hydroxydecanoic, 2-hydroxyisocaproic, and acetoacetic acids. Pathway analysis highlighted alterations in starch, sucrose, galactose, and amino sugar metabolism. PLS-DA model focused on genetic risk, revealed significant separation between High- and Low-Risk groups. Key metabolites include palmitic, mandelic, pyroglutamic, lactic, and ribonic acids. Altered pathways involved phenylalanine, tyrosine, tryptophan biosynthesis, as well as valine, leucine, isoleucine metabolism.

Conclusion: Untargeted metabolomic analysis demonstrated that patients exhibit progressive metabolic alterations according to the severity of the disease. Notably, a marked separation emerged between high- and low-genetic-risk groups, indicating potential for early diagnostics and stratified patient management.

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P05.025.B Genetic predisposition, dietary risk factors and intestinal microbiota in obese individuals with colorectal tumors

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Background: Genetic predisposition, intestinal dysbiosis, obesity, and an unhealthy diet are well-known risk factors for colorectal cancer (CRC).

Material and methods: To examine the interplay of these cofactors in colon carcinogenesis, we recruited 120 patients with colorectal polyps and collected blood samples for genetic analyses, mucosa-associated microbiota (MAM), lumen-associated microbiota (LAM), mucosa- and lumen-associated metabolome, and lifestyle/dietary habits (using validated EPIC questionnaires). Germline DNA from peripheral blood was analyzed by targeted-NGS using a custom panel of 107 genes involved in inherited cancer syndromes. MAM and LAM were analyzed by 16S rDNA sequencing. Metabolites were analyzed by bidimensional gas chromatography mass spectrometry. Patients were classified according to their body mass index and waist circumference in 75 normal-weight and 45 overweight/obese subjects.

Results: Twenty patients, 13 normal-weight and seven overweight/obese, carried germline pathogenic variants (PVs) in genes involved in colorectal cancer syndromes. No statistically significant difference was observed in genetic predisposition between normal-weight and obese patients. Dietary habits data revealed a statistically significant higher consumption of processed meat in obese patients. Both MAM and LAM 16S rDNA sequencing and mucosal and luminal metabolome analyses revealed different signatures in the two groups. Importantly, we found an enrichment of the pathogenic species *Finegoldia magna* in MAM of obese patients.

Conclusion: These data support the hypothesis that different risk factors may contribute to tumorigenesis in obese and normal-weight patients.

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P05.026.C Mitochondrial dysfunction in Alpha-1 antitrypsin deficiency models

***Sara Gil Martin**[1,2], Nerea Matamala[1], Carlos Benítez Buelga[1], Gema Gómez Mariano[1], Sheila Ramos del Saz[1], Marta Fernández Prieto[1], Juliana Manosalva[3], Diego Megías[3], Félix Docando[4], María C. Terrón[4], Javier Alonso García de la Rosa[1,2], Beatriz Martinez-Delgado[1,2]*

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Background: Alpha-1 Antitrypsin Deficiency (AATD) is a rare genetic disorder caused by mutations in the *SERPINA1* gene. The most common pathogenic mutation, known as the Z allele, results in the production of a dysfunctional AAT protein that accumulates as polymers within hepatocytes, leading to both lung and liver damage. The aim of this study is to investigate the metabolic differences induced by the Z allele in both cell models and liver organoids of AATD.

Material and methods: We studied a HepG2 cell line expressing the Z allele of *SERPINA1*, in parallel with a control HepG2 cell line, and hepatic organoids derived from both ZZ AATD patients and control individuals. These models of AATD were used to assess mitochondrial activity and integrity. Mitochondrial markers such as TOM20 antibody and MitoSOX were employed for immunofluorescence assays. Mitochondrial integrity was examined using transmission electron microscopy, and mitochondrial metabolism was evaluated through Seahorse metabolic assays.

Results: Both Z allele-expressing models (hepatic cell lines and organoids) exhibited an increase in mitochondrial mass, with altered mitochondrial activity under basal conditions. HepG2-Z cells and patient-derived organoids showed a significantly lower spare respiratory capacity compared to control HepG2 cells and organoids, which indicates mitochondrial dysfunction. Additionally, electron microscopy revealed irregular cristae and alterations in the mitochondrial lumen of both models.

Conclusion: The Z allele-expressing models show an alteration of the mitochondrial function and structure that could be related to the accumulation of AAT polymers within the cells. These alterations may contribute to the cellular dysfunction characteristic of AATD.

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P05.027.D Genotype-phenotype variability in adult cystic kidney diseases: evidence from seven cases

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Moldova, Republic of, [6]University of Medicine and Pharmacy Grigore T. Popa, Iasi, Romania, [7]Nephrology Clinic, Dialysis and Renal Transplant Center, Dr. C.I. Parhon University Hospital, Iasi, Romania.

Background: Cystic kidney diseases represent a group of disorders with overlapping phenotypes. While many are linked to primary cilium dysfunction (ciliopathies), others involve distinct non-ciliary mechanisms. This study examines seven clinical cases, focusing on genotype-phenotype correlations to guide personalized management approaches.

Material and methods: Seven cases from the Rare Renal Diseases Center in Iași, Romania, were analyzed using NGS (gene panels, whole-exome sequencing, or single-gene testing). The aim was to correlate genetic findings with clinical phenotypes and demonstrate how molecular analysis improves patient care.

Results: Pathogenic mutations were identified in four patients:

- *PKD1* associated ADPKD in a patient with bilateral polycystic kidney but no family history.
- *VHL*-related Von Hippel-Lindau disease with an increased risk of clear-cell carcinoma.
- Contiguous gene deletion syndrome involving *TSC2* and exons 31–46 of *PKD1* in a 50-year-old patient.
- X-linked Alport syndrome without hearing loss caused by a *COL4A5* mutation.

Key measures included: initiating Tolvaptan treatment for ADPKD; assessing the potential use of mTOR inhibitors and vasopressin receptor antagonists for *TSC2-PKD1* syndrome; implementing family screening for early prevention; providing genetic counseling for reproductive decisions. The patient with VHL was informed about a phase 2 clinical trial using Belzutifan.

Three additional cases involved missense variants of uncertain significance (*HNF1B, PKHD1, TTC21B*), highlighting the need for further studies to clarify pathogenicity.

Conclusion: This case series underlines the importance of genetic testing and phenotypic correlation in refining diagnoses, optimizing management, and guiding personalized therapies. These results emphasize the transformative potential of integrating molecular data into clinical practice of Nephrology.

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P05.028.A Comprehensive genomic and cytogenomic analysis reveals *C4B* deletion and mutation in wild-type *C4A* pediatric lupus nephritis: a case report of two siblings

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Background: Systemic lupus erythematosus (SLE) is a chronic, multifactorial disorder with variable clinical manifestations. Among the most common and serious manifestations is lupus nephritis (LN), affecting >50% of patients, with 20% progressing to end-stage renal disease.

Material and methods: We present the case of a sixteen-year-old girl and her younger sister, born to consanguineous parents. The older sister developed early-onset SLE at age 3 with LN, severe persistent hypocomplementemia (C3+C4), thrombotic microangiopathy, nephrotic syndrome relapses, arthralgia, and serositises, reaching ESRD and receiving a kidney transplant by age 13. The younger sister presented milder SLE starting at age 9 with hemolytic anemia, cutaneous and articular symptoms, normal renal function, normal C3, and low C4.

Results: NGS genetic testing revealed three VUS in *C4B* (p.L1120P, p.S1121C, and p.H1125=), previously reported in SLE patients and presumed inherited as a haplotype, in both sisters and their healthy parents. Via SNP-array analysis and qRT-PCR, a heterozygous de novo whole-gene *C4B* deletion was identified in the older sister, while *C4A*-loss-CNVs were excluded.

Conclusion: Deficiencies in early complement components are often present in SLE patients: although the specific association between lower total *C4* CNV, lower *C4A* CNV, lower *C4B* CNV, and SLE is a matter of debate, C4A- and C4B-null phenotypes often result from gene deletions. While the pathogenic role of the three variants is unconfirmed, the co-occurrence of a whole-gene *C4B* deletion and a complex haplotype with three VUS on the second allele appears compatible with the more prominent partial C4 deficit exhibited by the proband.

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P05.029.B Assessment of the clinical diagnostic potential of NGS-based panel tests in 100 cases with renal disease pre-diagnoses

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Background: This study aimed to determine the contribution of next-generation sequencing-based panel tests to clinical diagnosis by evaluating the genetic data of 100 patients who applied to the Division of Medical Genetics of Istanbul Faculty of Medicine with various renal disease pre-diagnoses between 2019 and 2024.

Material and sethods: DNA samples were extracted from the peripheral blood of patients. NGS analysis of the targeted gene panel was performed using the Nephropathies Solution Kit (Sophia Genetics) on the NovaSeq 6000 sequencing platform (Illumina). The exons and exon-intron boundary regions of 44 genes linked to renal disorders in the panel were sequenced. Segregation analyses were performed by Sanger sequencing. Sophia-DDM software was used to detect disease-related SNPs and CNVs.

Results: In 51% of cases, pathogenic (57.5%), likely pathogenic (6.3%), or variants of unknown clinical significance (VUS, 36.2%) were detected in targeted genes. Variant classification was performed according to the ACMG guidelines.

Conclusion: Many renal diseases exhibit genetic heterogeneity. Unfortunately, our panel test was limited to genes with the most frequently reported pathogenic variants in renal diseases.

According to our data, 49% of the cases were negative. This situation may be caused by a variation in another gene that is associated with the pre-diagnosis but is not included in the panel test. Therefore, we would like to emphasize that choosing a more comprehensive and powerful diagnostic test, such as CES/WES, instead of a panel test with limited gene content in diseases with genetic heterogeneity will significantly contribute to both rapid diagnosis and cost-effectiveness.

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P05.030.C Paternal *PEG10* deletion in a familial case of Silver-Russell Syndrome

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Background: Silver-Russell syndrome (SRS, MIM#180860) is an imprinting disorder, characterized by prenatal and postnatal growth retardation, relative macrocephaly at birth, prominent forehead, feeding difficulties, and body asymmetry. Clinical diagnosis is based on at least 4 out of 6 clinical signs (Netchine-Harbison clinical scoring system). The main molecular mechanisms are loss of methylation at the paternal *H19/IGF2:IG-DMR* (30–60%) localized in the 11p15 chromosomal region and maternal uniparental disomy of chromosome 7 (5–10%). While it is well known that deregulation of 11p15 imprinted genes (*IGF2*, *H19*, and *CDKN1C*) contribute to the SRS phenotype, those on chromosome 7 (*GRB10*, *PEG10*, and *MEST*) are still in discussion.

Case Reports: We report two brothers clinically diagnosed with SRS (postnatal growth delay, relative macrocephaly at birth, feeding difficulties, and SRS facies). One proband also has myoclonus dystonia and underwent growth hormone treatment.

Results: CGH-array revealed a deletion of 112 Kb including *PEG10* and *SGCE* genes, inherited from their unaffected father. Whole Exome Sequencing did not disclose other causative variants. PEG10 functions as a transcriptional repressor of cyclin-dependent kinase inhibitors, including *CDKN1C*, for which maternal gain-of-function variants are linked to SRS and IMAGe syndrome. Real-time PCR, performed on RNA from blood samples of probands and their healthy relatives, displayed a down-regulation of *PEG10* and an upregulation of *CDKN1C* expression only in the two brothers. Interestingly, *IGF2* was upregulated in the treated proband.

Conclusion: For the first time, we associate deregulation in the *PEG10* gene with the SRS phenotype, mediated by a gain-of-function effect on the *CDKN1C* expression.

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P05.031.D Molecular analysis of the *AGXT* gene in Moroccan patients with primary Hyperoxaluria Type 1: a study of 132 Cases

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Background: Primary hyperoxaluria type 1 (PH1) is one of the most common genetic diseases in the Moroccan population and is likely underdiagnosed. PH1 is an autosomal recessive disorder caused by a deficiency of alanine-glyoxylate aminotransferase due to a defect in the *AGXT* gene which contains 11 exons. The HGMD database reported 299 mutations. This study aims to identify recurrent mutations in Moroccan PH1 patients.

Material and methods: A series of 132 unrelated patients were recruited, based on clinical diagnosis, including elevated oxalate levels in blood and urine, and the presence calcium oxalate monohydrate (whewellite) of kidney stones confirmed through spectrophotometric analysis. Molecular studies were conducted using direct sequencing of recurrent mutations in the *AGXT* gene or through clinical exome sequencing.

Results: Eleven pathogenic mutations were identified, with the most frequent being *AGXT*(NM_000030.3): c.731T>C (p.Ile244Thr); c.697C>T(p.Arg233Cys) in exon 7 and c.976delG(p.Val326Tyrfs*15) in exon 10. Genetic analysis identified AGXT gene mutations in over 60% of cases. All mutations were found in exons 7; 10; 2 and 1 of the *AGXT* gene.

Conclusion: These findings support the development of a standardized diagnostic protocol, beneficial in low-to-middle incomes countries, including Morocco. Optimal treatment involves combined liver and kidney transplantation in addition to promising therapies based on RNA interference. This optimized approach enhances clinical management and provides a cost-effective framework for genetic counseling, benefiting both affected individuals and their families.

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P05.032.A A novel genetic variant in chronic intestinal pseudo-obstruction expands the *ACTG2*-related pathophysiological spectrum

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Background: Pathogenic variants in the *ACTG2* gene are linked to a spectrum of intestinal motility disorders ranging from congenital megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) to late-onset chronic intestinal pseudo-obstruction

(CIPO). However, the molecular mechanisms underlying these phenotypic differences remain unclear. This study aims to (1) characterize a novel missense variant of *ACTG2* in a familial case of late-onset CIPO and (2) compare its functional impact with the R178H mutation, which causes MMIHS.

Methods: Two adult male siblings with late-onset CIPO (ages 26 and 61 years) and their unaffected offspring underwent whole-exome sequencing (WES). Wild-type and mutant ACTG2 proteins were expressed in experimental models. Functional effects were evaluated by in silico modelling and confocal microscopy, comparing the new variant with wild-type and mutant R178H.

Results: WES identified a novel *ACTG2* c.209A>G (Y70C) variant in both affected siblings. In silico modelling predicted divergent behaviours for the two variants: Y70C is more prone to monomeric form, while R178H shows a tendency toward aberrant aggregation. Confocal microscopy confirmed these predictions, showing widespread monomeric actin G2 in cells transfected with Y70C plamids, while R178H induced G2 actin precipitation into amorphous aggregates.

Conclusions: the novel Y70C mutation induced misfolding of actin monomers, impairing their integration into filaments and aligning with loss-of-function behaviour consistent with the less severe late-onset phenotype. In contrast, the R178H mutation exerted a dominant-negative effect on the wild-type allele, promoting aberrant polymerization and bundle aggregation, altering the cytoskeletal architecture of enteric myocytes, consistent with the defective intestinal development observed in MMIHS.

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P05.033.B Autosomal recessive polycystic kidney disease and mimicking phenocopies in a retrospective Slovak single centre cohort

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Background: Autosomal recessive polycystic kidney disease (ARPKD) leads to early onset kidney failure and liver impairment. Biallelic pathogenic PKHD1 gene variants are mostly responsible for the disease. However, large genetic heterogeneity must be taken into consideration when ordering genetic analysis.

Material and methods: The aim of the study was to characterize retrospectively the manifestations, clinical course and the results of genetic testing in the group of patients with initial suspicion on ARPKD according to the renal ultrasound.

Results: The cohort of 29 patients was examined between the years 2012–2022. Manifestation was prenatal in 8 patients, neonatal in 13 and postnatal in 8 patients. Prenatal disease onset had the highest mortality, with the total mortality in the cohort reaching 20.7%. Genetic testing confirmed ARPKD in 22 patients, in negative cases the TMEM67, HNF1ß, NPHP3, NPHP4, and PKD1 gene variants were identified.

Biallelic protein truncating PKHD1 gene variants had perinatally lethal phenotype, representing the only known genotype-phenotype correlation in ARPKD. Patients with the NPHP-genes associated disease suffered from an equally severe condition, either. Conversely, a milder course was observed in TMEM67-associated disease, although renal failure and hepatic fibrosis occurred in the first decade. Disease due to PKD1 hypomorphic alleles was on the modest edge.

Conclusion: ARPKD is a severe disease with high mortality, renal and hepatic morbidity. In addition to the PKHD1 gene, many other genes produce a similar phenotype. Whole exome sequencing is an adequate diagnostic tool with a reliable result for genetic counseling in affected families.

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P05.034.C Highly variable expressivity of a CNV deletion involving *TBX4* in three siblings with lethal lung developmental disorder and their mildly affected mother and grandfather

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Background: In addition to pulmonary arterial hypertension and ischiocoxopodopatellar syndrome, single nucleotide variants (SNVs) and copy-number variant (CNV) deletions involving the dosage sensitive (pLI score = 1.0) T-box transcription factor *TBX4* gene have been associated with lethal lung developmental disorders (LLDDs) in neonates, including acinar dysplasia (AcDys) and congenital alveolar dysplasia (CAD). In contrast to missense SNVs, CNV deletions encompassing entire *TBX4* have been found exclusively as de novo events.

Material and methods: Whole-genome sequencing (WGS), RT-qPCR, luciferase reporter assay, computational analyses.

Results: We present a 3-generation family with three neonate siblings who died within 35–66 days after birth due to respiratory distress. Histopathological evaluation of the lung biopsy from one neonate showed pronounced alveolar simplification in the spectrum of CAD without hypertensive changes of the pulmonary arteries. WGS identified an ~ 108 kb CNV deletion encompassing *TBX4* in all three deceased infants. The deletion was also detected in their mother who has a history of pneumonia episodes and persistent thick upper airway secretions, and in the maternal grandfather who had surgically corrected genu valgum. RT-qPCR from the available lung biopsy showed a significant decrease in TBX4 expression. Computational analyses of the *TBX4* distant enhancer region in the affected and unaffected family members revealed 13 noncoding potentially hypomorphic SNVs, transmitted to the children from their father. We tested some of these variants using an in vitro luciferase reporter assay in fetal lung fibroblast cell line.

Conclusion: Our data further demonstrate complex compound inheritance of LLDDs and challenges in genetic counseling.

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P05.035.D Whole genome and transcriptome analysis of clonally expanded normal kidney cells from urines of ADPKD patients

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Capitanio[5], Andrea Salonia[5], Paola Carrera[6], Maria Teresa Sciarrone Alibrandi[2], Alessandra Boletta[3], Irene Franco[1]

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD), caused by inherited mutations in one allele of *PKD1* or *PKD2*, is characterized by progressive kidney cyst formation. Most cysts clonally expand from single cells that acquire somatic mutations in the wild-type copy of the *PKD* gene. We tested whether a higher rate of somatic mutation accumulation at the whole genome level might be a mechanism that favors cystogenesis in ADPKD.

Material and methods: We detected somatic mutations in single genomes by clonally expanding normal kidney cells from the urines of 6 ADPKD patients (aged 25–55) and 6 controls (aged 24–53). Clones were subjected to concomitant qPCR measurement of gene expression (n = 119), RNAseq (n = 16), and WGS (n = 46).

Results: High *PAX2* and *PAX8* expression revealed that all cultured clones originated from the kidney tubule epithelium, but the number of clones was higher (36 vs 8 colonies/urine sample, $p = 0.002$) and there was an upregulation of alanine-aspartate-glutamate metabolism in ADPKD vs controls. No somatic second hits in *PKD1* or *PKD2* were found in our normal cell clones. Somatic SNVs and indels linearly increased with age, with similar rates in both cohorts. However, a rare *VCAM1+* cell subset, described as mutation-prone in our previous study [1], was more prevalent in ADPKD (7.02% vs 0.89% of clones, $p = 0.003$). We will analyze somatic mutations in this subset.

Conclusion: In normal kidney cells from ADPKD patients, the age-related accumulation of somatic mutations is similar to control, but *VCAM1+* cells that may accelerate cyst growth are more common.

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P05.037.B Molecular findings in children with intrahepatic cholestasis

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Background: cholestatic liver disease is a condition that is commonly referred to in pediatric liver transplant centers. During the first months of life, inherited disorders are the second most common cause of cholestasis.

Material and methods: between 2017–2023 years, 213 pediatric patients (male- 117/45.1%) with suspected inherited intrahepatic cholestasis were evaluated by Sanger sequencing and/or NGS-sequencing (panel/WES/WGS/ WGS trio). The age of onset of cholestasis varies widely, from 0 months to 14 years, with a median age of 2 months [0;10].

Results: we identified disease-causing variants in 60% (128/213) of our study participants. Causative variants were identified in 39 different genes of monogenic diseases, top genes were: *JAG1* (Alagille syndrome; 21/128; 16.4%), *SERPINA1* (alpha-1 antitrypsin deficiency; 17/128; 13.3%), *ABCB11* (type 2 PFIC; 14/128; 10.9%), *ATP7B* (Wilson disease; 11/128; 8.6%), *AGL* (glycogenosis type 3; 7/128; 5.5%), *PKHD1* (polycystic disease type 4 (Karoli syndrome); 3.1%) and *ABCB4* (PFIC type 3; 4/128; 3.1%). In total, that genes account for more than half of the cases (60.93%) of monogenic forms of cholestasis syndrome. The majority of inherited cholestatic diseases are autosomal recessive (78.9%). Only 19.53% are monogenic diseases with an autosomal dominant inheritance pattern, and most of these are represented by Alagille syndrome.

Conclusion: in a single-center pediatric study, we identified monogenic variants in 39 known human's intrahepatic cholestasis genes. These findings support the usefulness of comprehensive multigene testing for diagnosing cholestasis and emphasize the evolving understanding of genetic variants contributing to the pathogenesis of this condition.

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P05.038.C Refining the diagnosis of Renal Hypouricemia: genetic and biochemical insights

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Background: Renal hypouricemia (RHUC) is caused by pathogenic mutations in *SLC22A12* and *SLC2A9*. Current clinic diagnostic criteria (SUA <2 mg/dL and FEUA >10%) exclude cases of mild hypouricemia (2<SUA < 3 mg/dL and FEUA >10%), which may be caused by monoallelic pathogenic mutations in these genes and could still pose health risks.

Material and methods: We studied individuals with suspected RHUC, identifying monoallelic and biallelic carriers of disease-causing mutations (DCMs). Additionally, for co-segregation and genotype-phenotype analysis, we recruited relatives of RHUC probands and additional individuals carriers of any DCM and not suspected of having RHUC. We ask for clinical data of all participants.

Results: Among 21 RHUC-suspected probands, 15 carried DCMs (71% diagnostic yield), including 3 biallelic and 12 monoallelic carriers. Ten distinct mutations were found, including 3 previously unlinked to RHUC. Many mutations were initially classified as VUS by automated classifiers but were reclassified as likely-pathogenic after incorporating published data, genotype-phenotype correlation and cosegregation results. Biochemical data revealed biallelic

individuals having SUA around 1 mg/dL and FEUA >20%. Monoallelic subjects showed SUA mostly between 2–3.3 mg/dL and FEUA >10%, consistent with mild hypouricemia. Significant SUA differences were found among non-carriers, monoallelic, and biallelic carriers.

Conclusion: Monoallelic carriers of *SLC22A12* or *SLC2A9* mutations may present with mild hypouricemia. The diversity of mutations found and the poor classification by automated classifiers highlight the need for specialized geneticists and personalized diagnostics. Revised diagnostic criteria, including adjusted SUA thresholds, are essential. Further research is needed to clarify the pathogenicity of mild hypouricemia and management strategies.

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P05.039.D Optimization of the diagnostic workflow for genetic liver diseases in pediatric and adult patients through Next Generation Sequencing

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Background: Genetic testing for liver diseases faces significant challenges, including variant interpretation and genotype-phenotype correlation. Moreover, limited evidence exists on its role in the diagnostic workflow of patients with hepatopathies. Clinical exome sequencing (CES) has emerged as a promising tool, offering flexibility and cost-effective workflows. This study aims at designing an algorithm to efficiently implement genetic testing in the diagnostic workup of hepatopatic patients.

Material and methods: We analyzed 197 pediatric and adult patients with liver disease referred to the Città della Salute e della Scienza Hospital, Turin, between July 2003-October 2024. CES was performed and analyzed using in silico gene panels. When available, pre-existing genetic reports were reviewed. Data were analyzed for diagnostic yield and stratified by patient characteristics, age and clinical suspicion.

Results: The cohort consisted of both pediatric and adult patients. Marked differences in diagnostic yield were observed between children and adults. Summarized data are reported in Table 1. In pediatric patients, genetic testing provided a high diagnostic rate and supported its use even for minor signs of liver disease. In contrast, genetic testing in adults showed limited utility, being most valuable for selected cases with unexplained liver disorders that had already undergone extensive routine testing without a diagnosis.

Conclusion: This study highlights CES as a valuable tool for diagnosing liver diseases, particularly in children. Additionally, the findings enabled the design of a diagnostic algorithm to guide genetic testing in liver diseases, optimizing diagnostic workflows and cost-efficiency.

Table 1. *Cohort description and result summary*.

FEATURE	PEDIATRIC	ADULTS	TOTAL
Demographics			
N. of patients, n (%)	154 (78.1%)	43 (21.2)	197 (100)
Age, median [IQR]	3 [0,10.8]	32 [21.5,53.5]	7 [1,16]
Male, n (%)	104 (67.5)	27 (62.8)	131 (67.2)
Non-Caucasian ethnicity, n (%)	28 (18.2)	2 (4.7)	30 (15.2)
Positive family history, n (%)	19 (12.3)	6 (14)	25 (12.8)
Transplanted patients, n (%)	59 (38.3)	5 (11.6)	64 (32.5)
Disease categories			
Cholestatic liver disorders, n (%)	58 (37.7)	16 (10.4)	74 (48.1)
Ciliopathies, n (%)	16 (10.4)	3 (1.9)	19 (12.3)
Metabolic disorders, n (%)	42 (27.3)	15 (9.7)	57 (37)
Syndromic disorders, n (%)	12 (7.8)	0 (0)	12 (7.8)
Unexplained disorders, n (%)	26 (16.9)	9 (5.8)	35 (22.7)
Diagnostic yield			
Overall, %	46.1	9.3	38.1
Cholestatic liver disorders, %	32.8	0	25.7
Ciliopathies, %	37.5	0	31.6
Metabolic disorders, %	73.8	13.3	57.9
Syndromic disorders, %	50	/	50
Unexplained disorders, %	34.6	22.2	31.4

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P05.040.A Expanded clinical phenotype of RINT1: infantile-onset recurrent acute liverfailure and neutropenia

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Background: Infantile-onset recurrent acute liver failure is a rare and life-threatening condition in pediatric patients, often triggered by febrile illnesses or infections. Genetic factors underlying RALF include mutations in genes involved in intracellular trafficking and autophagy. Variants in *RINT1*, a key component of the NRZ complex, have been implicated in RALF, but data on their clinical spectrum remain limited.

Material and methods: We present a case of an 8-year-old girl from the Chuvash population with RALF and neutropenia. Clinical data, biochemical evaluations, and genetic analyses were conducted, including whole-exome sequencing (WES) and 3D structural modeling of the identified *RINT1* variant (NM_021930.6:c.1435G>C, p.Ala479Pro). Population-specific analysis investigated the variant's origin.

Results: The homozygous *RINT1* variant was identified in the proband and associated with febrile and infection-induced liver failure episodes, elevated AST/ALT levels, hepatomegaly, and fibrosis. Neutropenia was consistently observed. Siblings with similar phenotypes succumbed to severe liver failure, supporting an autosomal recessive inheritance pattern. Structural modeling demonstrated that the p.Ala479Pro substitution disrupts alpha-helical stability, impairing RINT1 function. Population analysis revealed a shared ancestral origin within the Chuvash population.

Conclusion: This study expands the clinical phenotype of RINT1-related disorders to include RALF and neutropenia, emphasizing the importance of early genetic diagnosis and monitoring. The findings suggest a population-specific disorder with implications for genetic counseling and future therapeutic research. Functional insights into the mutation underscore the role of *RINT1* in vesicular trafficking, autophagy, and immune regulation, offering new avenues for understanding and managing RALF.

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P05.041.B MEFV mutational spectrum and clinical manifestations in Georgian FMF patients

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Background: Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disorder caused by mutations in the *MEFV* gene. Despite a known high carrier rate of 15% and its neighbourhood to endemic countries Armenia and Turkey, the overall situation concerning FMF in Georgia is still largely inextricable.

Material and methods: Over 300 Georgian subjects with FMF symptoms or/and family history have so far been recruited. Genotyping for twelve *MEFV* mutations plus three *SAA1* isoforms is performed using FMF-SAA1 StripAssay (ViennaLab Diagnostics). To date, full medical records and genotypes are available for 136 patients matching Tel-Hashomer criteria.

Results: 71 (52.21%) patients were female and 65 (47.79%) were male, with a mean age of 28.66 ± 16.63 years. We distinguished 16 different genotypes, M694V (66.44%), V726A (11.64%) and E148Q (8.90%) being the most frequent *MEFV* mutations. *SAA1* 1.1, 1.3 and 1.5 was present in 47.79%, 1.84% and 50.37% of alleles, respectively. Reported clinical manifestations included periodic attacks of fever (87.50%), abdominal pain (84.56%), arthralgia (71.32%), chest pain (45.59%), myalgia (44.12%) and skin rash (21.32%). A family history of FMF was reported by 50 (36.76%) subjects. Currently only 46 (33.82%) FMF patients receive colchicine therapy. Among the most striking observations was an extensive delay between the reported FMF onset (median: 9.00 years of age) and confirmed diagnosis (median: 25.50 years of age).

Conclusion: The *MEFV* mutational spectrum in Georgia is heterogenous, with similarities to neighbouring Armenia and Turkey. Awareness, as well as accessible diagnostic and therapeutic options for FMF, seem still insufficient and widely unavailable.

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P05.043.D Investigation of PNPLA3 rs738409 and TM6SF2 rs58542926 functional gene variants in patients with metabolic syndrome and non alcoholic fatty liver disease

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Background: This study aimed to investigate the relationship between PNPLA3-rs738409 and TM6SF2-rs58542926 functional gene variants with both genetic and clinical parameters in patient groups diagnosed with Metabolic syndrome (MetS) and Non-alcoholic fatty liver (NAFLD).

Material and methods: A total of 159 MetS, 120 NAFLD and 80 healthy individuals were included in the study. Variant analyses were performed using PCR-RFLP from genomic DNAs.

Results: While PNPLA3-rs738409 genotype frequencies did not show significant differences in both disease groups compared to controls, TT genotype of TM6SF2-rs58542926 variant was found to be higher in Mets patients. In the Mets group, the PNPLA3-rs738409-CC genotype was associated with increased platelet (PLT) count and high Diabetes Mellitus (DM) rate, the GG genotype with increased waist circumference and high free triodothyronine (fT3) levels, and the GC genotype with low weight and short stature. The TM6SF2-rs58542926-TC genotype was associated with decreased Alkaline phosphatase (ALP) levels. In the NAFLD group, the PNPLA3-rs738409-CC genotype was associated with high BMI, the GC genotype with advanced age, narrow leg/arm circumferences. The TM6SF2-rs58542926-CC genotype with weight gain and high blood pressure, TC genotype with low weight, low blood pressure and low gamma-glutamyl transferase (GGT) levels, and TT genotype was associated with narrow leg/arm circumference and high GGT levels.

Conclusion: Our study is the first to examine rs738409 and rs58542926 missense mutations together in MetS and NAFLD patients, and these results suggest that these functional gene variants may be important biomarkers in the etiopathogenesis of both MetS and NAFLD.

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P05.044.A Identification of type 2 diabetes-associated polymorphisms in DOCK7, ABCG8, UBE2E2, and SYN2 genes in the Uzbek population

Darya Zakirova[1], *Fazliddin Khonboev*[1], *Dilbar Dalimova*[1], *Guzal Abdullaeva*[2], *Feruza Takhirova*[3], *Anastasiya Punko*[1], *Nodira Alikhanova*[3], *Shahlo Turdikulova*[1], *Alisher Abdullaev*[1]

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Background: Diabetes is a leading cause of death, affecting nearly half a billion adults worldwide. With projections indicating a significant increase in prevalence, understanding the genetic factors contributing to diabetes, particularly type 2, is crucial.

Materials and methods: This study include 165 individuals: 125 patients with type 2 diabetes and 40 controls. PCR was performed using Real-Time PCR. A logistic regression analysis was conducted using the R programming language and the SNPassoc software package to identify statistically significant associations between genetic variants and disease risk under an additive genetic model.

Results: Genetic analysis showed an association between the rs4299376 variant and type 2 diabetes (OR:0.52, 95% CI: 0.32–0.84, $p = 0.008375$), as well as between the rs6780569 (OR:3.25, 95% CI: 0.99–10.64, $p = 0.021334$), rs7616006 (OR:1.8, 95% CI: 1.06–3.05, $p = 0.02316$), and rs12749263 (OR:0.50, 95% CI: 0.31–0.82, $p = 0.006540$) variants and type 2 diabetes. After Bonferroni correction, only rs4299376 and rs12749263 remained significant. Other gene variants rs7957197 and rs4402960 did not show an association with the disease ($p > 0.05$).

Conclusion: The findings underscore the importance of ethnic and geographical diversity in genetic studies and contribute to the understanding of type 2 diabetes in the Uzbek population. Further research is needed to explore the clinical implications of these genetic associations.

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P05.045.B Predicted loss of function variants in exons 20 and 21 of *OFD1* cause X-linked primary ciliary dyskinesia: a case report

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Background: Primary ciliary dyskinesia (PCD) is the most common motile ciliopathy characterized chiefly by early-onset sinopulmonary disease and variably present situs anomalies, subfertility, hydrocephalus, and congenital heart anomalies. PCD is genetically heterogenous; *OFD1* is an emerging cause of an X-linked recessive form. This gene encodes a pleiotropic protein found in basal bodies that functions in ciliogenesis and left-right patterning. Variants in this gene also cause Oral-Facial-Digital syndrome type 1, Joubert syndrome type 10, and retinitis pigmentosa type 23. To date, 15 individuals/kindreds with *OFD1*-related PCD have been reported and all have variants creating a premature termination codon (PTC) in exons 20 or 21 (total of 23).

Methods: Commercial NGS-based testing of 47 PCD-related genes was performed on proband peripheral blood DNA.

Results: We report an 8-year-old boy with recurrent sinopulmonary infections (pneumonia, sinusitis, and otitis media), chronic wet cough, and situs inversus consistent with PCD. He is neurodevelopmentally typical and presents with mild dysmorphisms including hypertelorism and brachydactyly. Genetic testing revealed a likely pathogenic de novo hemizygous *OFD1* c.2725C>T, p.(Arg909*) variant. This variant was reported previously in a family with two affected males with PCD.

Conclusion: These findings provide further clinical evidence that X-linked PCD belongs in the phenotypic spectrum of *OFD1*. A review of reported cases establishes a clear correlation with PTC-causing variants in exons 20 or 21 which were previously shown to escape nonsense-mediated decay. Additional variable features include intellectual disability, macrocephaly, obesity, polydactyly and distinctive facial features. Importantly, similar variants in these exons also cause Joubert syndrome.

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F. Chowdhury: None. **A. Marwaha:** None.

P05.046.C A case of gastrointestinal defects and immunodeficiency syndrome-1 (GIDID1) in a 3-month-old Bulgarian girl with two variants in *TTC7A* gene: diagnostic challenges and implications

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Background: Gastrointestinal defects and immunodeficiency syndrome-1 (GIDID1) is a complex rare autosomal-recessive disorder with a range of intestinal abnormalities (including apoptotic enterocolitis, intestinal atresias at multiple locations throughout the small/large intestines, intestinal stenosis), along with immune deficiency. This condition is associated with pathogenic variants in the tetratricopeptide repeat domain 7A (*TTC7A*) gene; homozygous state is correlated with poor prognosis and short life expectancy. Genetic testing for *TTC7A* gene abnormalities associated with GIDID1 is advised for patients with multiple gastrointestinal issues and frequent infections in order to offer effective treatment, prenatal screening, and genetic counseling. We present a case of a 3-month-old girl diagnosed with intestinal atresia, severe hypotrophy and chronic diarrhea, with a suspected diagnosis of congenital enteropathy.

Material and methods: Genomic DNA was extracted from peripheral blood of the proband and both parents and analyzed using whole exome sequencing (WES).

Results: WES analysis revealed two heterozygous variants in the *TTC7A* gene: c.315_318del, p.(Tyr105Ter) and c.1027G>A, p.(Glu343Lys) - classified as pathogenic and a variant of uncertain significance (VUS) in the ClinVar database, respectively. Sanger sequencing and segregation analysis in the family revealed one paternally and one maternally inherited variant, which validated the suspected diagnosis GIDID1.

Conclusion: This case highlights the critical role of genetic testing for complex congenital disorders like GIDID1. Considering the significant morbidity and mortality associated with this condition, clinicians should maintain a high level of awareness

to recognize this syndrome and to be familiar with the benefits of genetic testing due to the treatment scarcity.

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P05.047.D Incidental findings in HHT-related genes in prenatal unindicated exome sequencing: implications of reclassification

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Background: Hereditary Hemorrhagic Telangiectasia (HHT) is a highly penetrant vascular disorder that can lead to severe complications, many of which are preventable through proactive surveillance. Since 2013, the American College of Medical Genetics and Genomics (ACMG) has provided guidelines for reporting secondary findings (SF) in clinical exome and genome sequencing. In May 2021, HHT-related genes ACVRL1 and ENG were incorporated into ACMG SF v3.0, while SMAD4 had been included earlier due to its link with juvenile polyposis.

Material and methods: We reviewed data from the HHT National Center, focusing on families in which incidental HHT-related variants were identified during unindicated prenatal trio-exome sequencing.

Results: HHT-related variants in ACVRL1 and ENG were identified in 15 individuals across five families. The testing was conducted in various laboratories. Initially all five variants were considered potentially disease-causing and were classified as either warm VUS, likely pathogenic or pathogenic. Following the 2024 ClinGen Hereditary Hemorrhagic Telangiectasia Expert Panel Specifications, all five variants were downgraded to VUS. Clinical findings ranged from asymptomatic presentations to epistaxis and/or telangiectasias, but no individuals had known AVMs or met the Curaçao criteria at the time of molecular diagnosis.

Conclusion: We anticipate a growing number of prenatal exome tests as the test becomes more feasible, underscoring the importance of accurate variant classification. Genomic variants identified in asymptomatic population have a higher chance of being false positives. Adherence to the latest ClinGen guidelines assists in classification of variants and the appropriate management of individuals with HHT.

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P05.048.A Homozygous pathogenic variant c.922G>A in the *CTNS* gene: a recurrent mutation in Moroccan patients with nephropathic cystinosis

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Background: Nephropathic cystinosis is a rare autosomal recessive lysosomal storage disorder characterized by an accumulation of cystine in the lysosomes leading to progressive renal failure and extra-renal comorbidity. It is caused by pathogenic variants of the cystinosin gene (*CTNS*). The spectrum of mutations in the *CTNS* gene is not well defined in Moroccan population.

Material and methods: Here, we investigated five families with at least one child affected by nephropathic cystinosis. Molecular analysis was performed by clinical exome sequencing in two families and by targeted Sanger sequencing of the *CTNS* gene in three families.

Results: We report a known mutation c.922G>A in these families which seems to be a recurrent mutation in the Moroccan population. This mutation was first found in two families by clinical exome sequencing. This result allowed us to search for this mutation by Sanger sequencing in the three other families.

Conclusion: We propose first line genetic testing based on screening of the c.922G>A mutation in the *CTNS* gene. This strategy will allow cost-effective diagnosis confirmation as well as early administration of treatment with oral cysteamine and will improve genetic counseling in families.

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P05.049.B Xq26.3 Microduplication as a Genetic Cause of Pituitary Macroadenoma and Gigantism

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Background: Excessive growth hormone (GH) levels before the closure of the epiphyseal plates lead to gigantism, which is characterized by tall stature. The most common cause is GH-producing pituitary adenomas, while pituitary hyperplasia is less frequently observed. In a study by Trivellin et al. (2014), 43 nonsyndromic gigantism cases without known genetic causes associated with pituitary tumors, were examined. Among them, 14 patients with gigantism in early childhood and symptoms in late infancy were found to have microduplications in the Xq26.3 region. Subsequent studies identified similar cases, leading to the classification of X-linked acrogigantism (XLAG; OMIM #300942). XLAG is an X-linked dominant disorder characterized by advanced bone age, accelerated growth velocity, tall stature, and elevated GH, IGF-1, and prolactin levels. We present a 5-year-old girl with gigantism and a pituitary macroadenoma, in whom a microduplication in the Xq26.3 region was detected.

Methods: Microarray analysis was performed.

Results: Microarray analysis revealed a 606 kb microduplication in the Xq26.3 region, encompassing the ARHGEF6, CD40LG, RBMX, and GPR101 genes. *GPR101* (OMIM *300393) is known to be associated with growth hormone-secreting pituitary adenomas. The alteration was classified as pathogenic and considered to clarify the patient's symptoms.

Conclusion: In this study, we present a patient diagnosed with XLAG resulting from Xq26.3 microduplication. Chromosome Xq26.3 microduplication syndrome associated with pituitary adenoma should be considered in patients with early growth hormone elevation and significant height increase.

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P05.050.C Genotype-phenotype correlation in patients with 17q12 microdeletion and *HNF1B* point alterations

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Background: The *HNF1B* gene encodes a transcription factor crucial for the development of multiple organs, including kidneys, pancreas, liver, intestines, and neurons. It regulates key biological processes such as nephrogenesis, ion transport, and cellular polarity, among others. Pathogenic *HNF1B* variants disrupt these functions, leading to a broad spectrum of renal and extrarenal manifestations. Patients can be classified into three groups based on their genetic alteration: 17q12 microdeletion, truncating point mutations, and missense point mutations. This study aims to establish genotype-phenotype correlations for *HNF1B* variants in a closed cohort predominantly from Galicia (region in western Europe characterized by unique geographical, ethnic, and consanguinity patterns, which enhance the genetic value of this population).

Material and methods: This retrospective, observational, multicenter study analyzed a large cohort of patients with suspected hereditary kidney diseases. Genetic analysis was conducted via Next-Generation Sequencing (NGS) and/or Sanger sequencing. 31 individuals (27 probands, 4 relatives) carrying *HNF1B* variants were selected, and detailed clinical data were collected and analyzed.

Results: Among probands, 55.55% carried the 17q12 microdeletion, 18.52% truncating mutations, and 25.93% missense mutations. Over 60% of probands presented renal cysts, hyperechogenicity, and/or hypomagnesemia. Other manifestations observed were hyperuricemia, Congenital Anomalies of the Kidney and Urinary Tract (CAKUT), diabetes/Maturity-Onset Diabetes of the Young (MODY), and hepatic or neurological anomalies. Clinical features varied among genetic variants.

Conclusion: This study highlights the broad and variable multisystemic impact of *HNF1B* variants, reinforcing the need for accurate genetic diagnosis to guide personalized genetic counseling and patient management based on the specific mutation type.

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P05.051.D A novel *COL4A1* truncating variant in an Italian family mimicking Alport syndrome

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Background: Alport syndrome (AS) is the prevalent genetic diagnosis in patients with microhematuria and chronic kidney disease (CKD). However alternative monogenic disease should be considered in genetically unresolved patients. Rare *COL4A1* missense variants are associated with Hereditary angiopathy, nephropathy, aneurysms, and muscle cramps (HANAC syndrome) characterized by renal cysts, thus this gene is not routinely investigated in suspicion of AS. Intriguingly, here we describe a novel truncating *COL4A1* variant in an Italian family with AS-phenotype, vascular malformation without renal cysts.

Material and methods: The proband was diagnosed with AS given the association of microhematuria, hearing loss and CKD. Similar features were observed in other relatives and a brother underwent renal biopsy revealing thin glomerular basement membranes overlapping the pattern observed in AS. Pathogenic variants in *COL4A3-A5* genes were not identified and the family was genetically unresolved since the son of the proband underwent Whole Exome Sequencing (WES) analysis for congenital spastic paraplegia identifying a heterozygous pathogenic truncating *COL4A1* variant in NC1 domain (c.1186C>T, p.Arg396Ter)

Results: Segregation analysis revealed *COL4A1* p.Arg396Ter variant in all affected relatives: mother, sister, aunt, uncles. Moreover, genotype-driven further clinical assessments delineated in the family extra-renal clinical features related to *COL4A1*-disease, i.e. cerebrovascular and retinal vascular malformations and elevated CK levels.

Conclusion: This report add insights into *COL4A1*-spectrum disease and usufulness of wide genetic approach. *COL4A1* gene should be investigated in genetically unresolved AS patients. The genetic diagnosis is even more crucial for accurate clinical management preventing misdiagnoses and to guide managment of extrarenal manifestations.

C.O.I. Disclosure:

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P05.052.A In Pursuit of a novel variant in LIPI gene: a rare case of hypertriglyceridemia and hepatosteatosis

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Background: *LIPI* gene, located on chromosome 21q11.2, plays a crucial role in maintaining lipid homeostasis. Genetic studies suggest that *LIPI* mutations may lead to dyslipidemia in humans and hypertriglyceridemia in mice. Presented case is a 27-year-old male who was admitted with elevated triglyceride levels and subsequently developed hypertriglyceridemia and hepatosteatosis during follow-up. Genetic analysis identified a novel, likely pathogenic variant in *LIPI*.

Material and methods: DNA was isolated from peripheral blood leukocytes obtained from the patient, and a clinical exome panel covering 6380 disease-associated genes was analyzed. The

analyses were performed using the Illumina NextSeq platform with the SOPHIA Clinical Exome Solution (CES) v3.

Results: The c.335A>G (p.Asp112Gly) missense variant was identified at heterozygous state in *LIPI* and classified as likely pathogenic based on ACMG criteria (PM2, PP3_Strong). Human Splicing Finder (HSF) analysis suggested potential splicing alterations due to changes in the ESE/ESS motif ratio and cryptic donor site activation. The variant's REVEL score of 0.957 further supports its high pathogenicity potential. When WT and D112G structures were examined using molecular dynamics methods, findings were obtained that the D112G change affected the protein function.

Conclusion: To the authors knowledge, this is the first hypertrigliseridemia case with a novel mutation in *LIPI*. Dyslipidemia is a major public health risk and a leading cause of preventable mortality. This case underscores *LIPI*'s role in lipid metabolism and dyslipidemia. Given the limited genetic studies on *LIPI*, further research is needed to expand its genetic spectrum and develop new therapeutic approaches.

C.O.I. Disclosure:

N. Sandal Filikci: None. **Z. Manav Yigit:** None. **M. Unal:** None.

P05.054.C Novel ATP7B variants in pediatric hepatic Wilson disease from Northern India

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Background: Most ATP7B mutation variants found globally do not match the Indian population, possibly due to the mixed anthropology and ethnic diversity. The hepatic manifestation of Wilson disease (WD) is an aggressive phenotype in children. Due to the paucity of exclusive literature, we aimed to assess the novel variants of ATP7B mutation and its correlation with the hepatic phenotype.

Material and methods: Children with hepatic WD diagnosed by modified Leipzig criteria having bona fide symptoms, on chelators underwent molecular confirmation by whole-exome sequencing. Known and novel variants were identified.

Results: 50 WD patients (aged 15.2 ± 4.5 years, 37 boys) had 71 ATP7B variants (45 heterozygous, 26 homozygous), located on exons 2(32%), 18 (13%), 8(10%), 13(10%) and 14(9%). Most common variants were c.813C>A(p.C271X) and c.3809A>G(p.N1207S). Deep intronic changes were found in three patients. The presentation was chronic hepatitis (64%), decompensation [ascites and/or encephalopathy] (26%) and acute liver failure (10%) with an overall mean pediatric end-stage liver disease (PELD) score of 11.4 ± 9.2. Of the entire cohort, 15 novel variants were identified in 11 patients (Table 1) with 100% endogamy or third-degree consanguinity. Of this subset, three patients had one variant each that was known pathogenic. Over a follow-up of 7.1 ± 3.8 years, six and three patients developed renal tubular dysfunction and neurological involvement respectively. Liver dysfunction improved in all.

Conclusion: Novel ATP7B variants are seen in 21% of Northern Indian cohort. Though liver functions recover on chelation, other systemic phenotypes may ensue in follow-up

C.O.I. Disclosure:

A.K. Mishra: None. **M. Sen Sarma:** None. **A. Dubey:** None. **A. Srivastava:** None. **U. Poddar:** None.

Table 1. Natural history and outcome of the novel ATP7B variants.

Patient number	Age at diagnosis (years)	Sib death	Presentation	Years of follow-up	Added problems at follow-up	Exon/ Intron Number	Zygosity	Standard nomenclature	Genomic Nomenclature	Type of variant	Known or novel variant
1	7	Yes, two	CH	12	None	E7	Hom	c.2072G>A (p.G691E)	chr13:g.52534333C>T	M	N
2	9	No	CH	4	RTD	E13	Het	c.2951C>T (p.P984L)	chr13:g.52520529G>A	M	N
						E17	Het	c.3626T>C (p.L1209P)	chr13:g.52513260A>G	M	N
3	12	No	DC	1.5	BM toxicity to D-pencillamine	E18	Het	c.3809A>G (p.N1270S)	chr13:g.52511706T>C	M	KP
						E18	Het	c.2339T>C (p.L780P)	chr13:g.52532463A>G	M	N
4	3	Yes, two	CH	12	Fatty liver, NI	E7	Hom	c.2072G>A (p.G691E)	chr13:g.52534333C>T	M	N
5	8	No	DC	4	None	E16	Het	c.3556G>A (p.G1186S)	chr13:g.52515217C>T	M	N
						E17	Het	c.3601G>A (p.E1201K)	chr13:g.52513285C>T	M	N
6	10	No	DC	10	RTD	I16	Het	c.3557-8C>A	chr13:g.52513337G>T	SR	N
						E8	Het	c.2339T>C (p.L780P)	chr13:g.52532463A>G	M	N
7	10	No	DC	10	Large gastric varices, NI	E10	Hom	c.2522T>G (p.V841G)	chr13:g.52524461A>C	M	N
8	13	No	CH	9	RTD	E11	Het	c.2668G>T (p.V890L)	chr13:g.52524205C>A	M	KP
						E8	Het	c.2312_2313del (p.F771Cfs*23	chr13:g.52532490_52532491del	F	N
9	13	No	CH	1.5	RTD, NI	E14	Het	c.3188C>G (p.A1063G)	chr13:g.52518300G>C	M	N
						E20	Het	c.4112_4114del (p.L1371del)	chr13:g.52509740_52509742del	ID	N
10	8	No	DC	10	RTD	I16	Het	c.3557-8C>A	chr13:g.52513337G>T	SR	N
						E8	Het	c.2339T>C (p.L780P)	chr13:g.52532463A>G	M	N
11	11	No	DC	2	RTD	I3	Hom	c.1544-3C>G	chr13:g.52542746G>C	SR	N

CH: chronic hepatitis, DC: decompensated, RTD: renal tubular dysfunction, BM: bone marrow, NI: neurological involvement, E: exon, I: intron, Hom: homozygous, Het: heterozygous, M: missense, SR: splice region, ID: inframe deletion, F: frameshift, N: novel, KP: known pathogenic

P05.055.D Genome analysis of situs inversus with and without primary ciliary dyskinesia

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Background: Situs inversus (SI) or left-right mirror reversal of the visceral organs is a developmental condition occurring in roughly 1 out of every 6000 to 8000 people. SI can occur together with the recessive disorder Primary Ciliary Dyskinesia (PCD) in the context of disrupted motile ciliary function in the early embryonic node, during specification of the left-right axis. However, most people with SI do not have PCD, and the etiology of their condition remains poorly studied. Although a general reversal of functional brain asymmetry in SI was not observed, they showed a marginally increased prevalence of atypical lateralization [1].

Material and methods: We previously sequenced the genomes of 15 people with SI, of which 6 had PCD, as well as 15 unaffected controls [2]. Now, we have sequenced the genomes of a further 23 SI individuals (of which 2 have PCD) and 23 controls.

Results: In the first wave of data collection, we found that while the 6 people with SI and PCD all had likely causal recessive mutations in genes already known to cause PCD, we only found 4 out of the 9 non-PCD SI cases had likely causative mutations.

Conclusion: We are now analyzing the new 23 SI individuals and 23 controls and will integrate with the first wave to better understand the genetic contributions to non-PCD SI and their possible relevance to brain asymmetry. This may provide clues to developmental mechanisms of left-right axis formation beyond motile cilia at the embryonic node.

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M. Wang: None. **S.E. Fisher:** None. **G. Vingerhoets:** None. **C. Francks:** None.

P05.056.A Longer leukocyte telomere length increases the risk of lung injury in children with COVID-19

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Background: The global pandemic of COVID-19 caused a significant impact on society, and emphasized the need to understand the factors that determine individual susceptibility to various forms of the disease. The susceptibility factors to lung injury of COVID-19 are not yet fully understood. Leukocyte telomere length (LTL) is sensitive to reactive oxygen species damage and inflammatory processes. Our study aimed to investigate the association between LTL and lung injury among children with COVID-19.

Material and methods: The relative average length of chromosome regions was studied in the groups of children (n = 210) with COVID-19. The analysis uses a method of multiplex monochrome quantitative real-time PCR on DNA samples isolated from the peripheral blood leukocytes.

Results: 210 children were divided into 2 groups: group 1 - children with lung injury (all types of pneumonia) (n = 156), group 2 - without injury (n = 54). Among children with lung injury during COVID-19 were detected statistically significantly longer telomere length according to the nonparametric Mann-Whitney test, compared to children without lung damage ($p < 0.05$ $p = 0{,}0196$).

Conclusion: It is one of the first studies to evaluate the role of LTL as a predictive biomarker in pediatric patients with COVID-19. In the current study, the association of longer relative telomere length with lung injury comparatively to shorter telomere in children without lung injury has been demonstrated. Thus, relative telomere length can be proposed as a risk marker of lung injury in children with COVID-19.

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P05.057.B First report of a novel *FLNA* long isoform missense mutation causing late-onset Chronic Intestinal Pseudobstruction (CIPO)

Ilaria D'Amato[1], *Enrico Ganguzza*[2], *Guido Basilisco*[2], *Alessia Strippoli*[1], *Elkadia Mehmeti*[1], *Erika Salvi*[1], *Maurizio Vecchi*[2,3], *Giuseppe Lauria*[1,4], *Margherita Marchi*[1]

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Background: CIPO is a rare intestinal motility disorder that can be associated with systemic diseases or genetically determined. X-linked CIPO (CIIPX) results from *FLNA* gene mutations. This gene encodes filamin A, which is crucial for cytoskeletal integrity and smooth muscle function. Previous CIPO cases linked to *FLNA* involved early-onset and often extra-intestinal manifestations.

Material and methods: Following a comprehensive clinical assessment of the proband, genomic DNA was subjected to WES analysis; variant segregation analysis within the family was conducted using Sanger Sequencing.

Results: A 36-year-old male presented with late-onset CIPO characterized by progressive constipation, vomiting, and weight loss. Genetic screening through WES was performed. Sequencing analysis in the patient revealed a hemizygous c.56G>T variant in the long-isoform of the *FLNA* gene (NM_001110556.2). Sanger sequencing confirmed the variant and demonstrated its segregation within the family: heterozygous in the mother and absent in the father and brother. This novel missense variant (p.Gly19Val) was classified as VUS according to ACMG guidelines.

Conclusion: This study reports the first case of late-onset CIIPX associated with a novel missense mutation (p.Gly19Val) in the *FLNA* gene. Located exclusively within the long isoform of filamin A, predominantly expressed in intestinal smooth muscle, this variant highlights the crucial role of this isoform in intestinal motility. Our findings expand the phenotypic spectrum of filaminopathies and provide insights into the pathogenesis of late-onset genetic CIPO.

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P05.058.C Alport syndrome associated with a novel COL4A3 variant: clinical and genetic findings

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Background: Alport syndrome (AS) is caused by mutations in type IV collagen genes, leading to hematuria, progressive renal failure, hearing loss, and ocular abnormalities. Most cases have mutations in *COL4A5* (X-linked), while mutations in *COL4A4* and *COL4A3* cause the autosomal recessive form and, rarely, the autosomal dominant form. In this study, we report the variable clinical manifestations in seven individuals from the same family with consanguineous marriages, including four homozygous and three heterozygous carriers of a novel *COL4A3* variant.

Methods: Genetic analysis was conducted using next-generation sequencing and targeted variants were analyzed by Sanger sequencing.

Results: Seven patients were examined, four homozygous and three heterozygous for a novel, likely pathogenic c.2347_2370del variant in *COL4A3*. No ocular lesions were identified in any of the patients. Homozygous individuals, diagnosed at ages ranging from 2 to 8 years, all exhibited with early-onset nephrotic syndrome and hearing loss in two of them. Among the heterozygous individuals, one had been monitored for hematuria since age 10. Segregation analysis was performed on the parents of a patient, revealing that both were heterozygous carriers. Subsequent clinical evaluation detected microscopic hematuria in the mother (aged 31), whereas no renal pathology was observed in the father (aged 38). These findings suggest a more severe clinical course in homozygous individuals compared to heterozygous carriers.

Conclusion: This study provides additional evidence for the expanding phenotypic spectrum associated with the novel *COL4A3* variant, contributing to a better understanding of its clinical variability and implications for patient management.

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P05.059.D Targeted sequencing of dyshormonogenesis-associated genes in congenital hypothyroidism cases with gland-in-situ from North Macedonia

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Background: The prevalence of congenital hypothyroidism (CH) in the Republic of North Macedonia is approximately 1 in 1,670 live births, with over fifty percent presenting as "gland-in-situ" (GIS CH). GIS CH includes CH cases with dyshormonogenesis; however, the proportion of GIS CH cases harboring mutations in CH-associated genes has rarely been investigated. This research represents the first investigation into the genetic basis of CH in Macedonia.

Material: A phenotype-driven approach was used to screen known dyshormonogenesis-associated genes (TG, TPO, DUOX2, DUOXA2, SLC26A7, SLC5A5 (NIS), IYD, and TSHR in GIS cases and TSHR and PAX8 in cases with thyroid hypoplasia. The cohort included non-syndromic, iodine-sufficient CH cases ($n = 40$) exhibiting a range of biochemical thyroid dysfunction ranging from severe permanent to mild transient and subclinical CH, along with 11 familial cases.

Results: All CH cases were term-born, with birth weight >3,000 g, and thyroid morphologies included goiter ($n = 11$), thyroid hypoplasia ($n = 6$), and apparently normal-sized thyroid. Potentially pathogenic variants were identified in 14 cases, of which four were definitively causative. Oligogenic variants were detected in three cases. Seventeen variants (nine novel) were identified in TPO ($n = 4$), TG ($n = 3$), TSHR ($n = 4$), DUOX2 ($n = 4$), and PAX8 ($n = 2$) genes. No mutations were detected in DUOXA2, NIS, IYD, and SLC26A7.

Conclusion: The low mutation frequency observed in our cohort suggests that factors beyond established genetic causes, such as environmental influences or novel genetic etiologies, may contribute to the pathogenesis of CH.

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N. Zdraveska: None. **V. Anastasovska:** None. **M. Kocova:** None.

P05.060.A Effects of long-term glucose and fructose consumption on liver ageing and Epigenetic changes

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Background: Calorie restriction is well-known for slowing ageing and extending lifespan. White sugar, composed of glucose and fructose, is processed differently in liver cells. Glucose metabolism is tightly controlled by the enzyme phosphofructokinase-1 (PFK-1), while fructose bypasses this regulation. Consuming glucose and fructose together for a long time may promote an anabolic state, leading to senescence in liver cells.

Material and methods: Standard chow diet-fed rats were divided into four groups: a control group (water), a glucose group (20% glucose), a fructose group (20% fructose), and a mixed group (10% glucose + 10% fructose). Blood biochemical values, weight gain were measured and open field and rotarod tests were performed. Liver steatosis and senescence were assessed using hematoxylin-eosin and s-β-galactosidase staining. Expression and methylation-specific PCR analyses were performed for the genes related to ageing and metabolism.

Results: The glucose group gained the most weight and had the best performance in the open field test, while the mixed group had the poorest results in the rotarod test. Triglyceride levels were significantly higher in the glucose and mixed groups. Steatosis and cell senescence were only observed in the fructose and mixed groups. Methylation analysis revealed increased FOXO4 methylation in the mixed group, while *PKLR*, *PFKL*, and *DNMT1* methylation levels were elevated in both the fructose and mixed groups.

Conclusion: We found that prolonged glucose+fructose consumption leads to liver fat accumulation and cellular senescence, but methylation analyses did not fully explain the underlying mechanisms. Further research is needed to uncover the molecular pathways involved.

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P05.061.B Is LCP1 *rs7324845*gene variant related to obesity or NAFLD in adolescents

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Background: Lymphocyte Cytosolic Protein 1 (LCP1) is one of the important genes under investigation in NAFLD, but these studies are limited in adolescents. This study aimed to investigate the relationship between the LCP1 gene and obesity-NAFLD in adolescents.

Material and ethods: We included 86 obese and 45 healthy adolescents. We assessed hepatostatosis with ultrasonography and measured anthropometric features. Minisequencing was performed for LCP1 rs7324845 variant using genomic DNA isolated from samples and performed with detection primers using SnaPshot Multiplex Ready Reaction Mix and run on an automated capillary electrophoresis. Multiplex peaks were analyzed with GeneMapper 5 Software. We used Bayesian Kendall's tau test to assess the evidence for H0 (independence) and H1 (correlation) hypothesis, and frequentist statistics.

Table 2. Bayesian Kendall's tau test between groups.

	Obese vs Control		Between 3 groups [NAFLD (+) vs NAFLD (−) vs Control]		NAFLD (+) vs NAFLD (−)		LCP1 rs7324845 Gene Variants (AA vs AG vs GG)	
	Bayesian Kendall's tau	BF_{10}	Bayesian Kendall's tau	BF_{10}	Bayesian Kendall's tau	BF_{10}	Bayesian Kendall's tau	BF_{10}
LCP1 rs7324845 Gene variant	−0.094	0.316	0.102	0.499	_	_	—	
NAFLD	1.000	6.58E+37	0.882	3.20E+46	—		−0.094	0.316
Sex	0.142	0.872	0.054	0.174	0.142	0.872	0.083	0.264
Age (month)	0.147	1.006			0.147	1.006	−0.005	0.142
Weight SDS	0.068	0.215	0.113	0.688	0.068	0.215	−0.097	0.327
Height SDS	−0.097	0.333	0.571	4.98E+18	−0.097	0.333	0.048	0.174
BKI SDS	0.048	0.174	0.072	0.237	0.048	0.174	−0.040	0.164
WC SDS	0.165	1.501	0.581	3.75E+19	0.165	1.501	−0.085	0.272
Sistolic TA SDS	0.025	0.154	0.626	7.46E+21	0.025	0.154	0.030	0.157
Diastolic TA SDS	0.152	1.06	0.265	1291.375	0.152	1.06	0.030	0.156
Birth weight SDS	0.009	0.153	0.257	813.831	0.009	0.153	−0.094	0.3
Weight gained by mother during pregnancy	0.070	0.233	−0.008	0.125	0.070	0.233	0.075	0.243
Glucose	−0.114	0.453	0.114	0.457	−0.114	0.453	−0.023	0.149
HbA1c	−0.059	0.196	−0.019	0.121	−0.059	0.196	−0.007	0.146
Insulin	0.121	0.509	−0.003	0.12	0.121	0.509	−0.005	0.145
Fructoseamine	−0.020	0.175	0.425	4.35e0+8	−0.020	0.175	−0.093	0.287
HOMA-IR	0.104	0.371	0.002	0.163	0.104	0.371	−0.033	0.159
AST	0.151	1.063	0.386	9.89e0+6	0.151	1.063	−0.017	0.147
ALT	0.189	3.326	0.079	0.271	0.189	3.326	0.044	0.169
GGT	0.354	3626.052	0.389	1.26e0+8	0.354	3626.052	−0.022	0.155
Total bilirubin	−0.147	0.76	0.519	1.18E+13	−0.147	0.76	−0.034	0.171
Cholesterol	0.122	0.536	−0.212	16.378	0.122	0.536	−0.059	0.194
Triglicerid	0.112	0.439	0.033	0.135	0.112	0.439	0.113	0.446
LDL	0.148	1.012	0.202	30.904	0.148	1.012	−0.031	0.155
HDL	−0.113	0.444	0.083	0.301	−0.113	0.444	−0.087	0.282
creatinin	0.009	0.144	−0.246	462.331	0.009	0.144	−0.002	0.143
BUN	−0.151	1.061	0.114	0.697	−0.151	1.061	−0.006	0.144
Albumin	0.049	0.177	0.011	0.118	0.049	0.177	−0.036	0.162
Free T4	0.006	0.148	0.080	0.275	0.006	0.148	0.039	0.167
TSH	0.044	0.173	−0.010	0.121	0.044	0.173	0.106	0.371
Cortisole	0.156	0.514	0.168	4.467	0.156	0.514	−0.053	0.238

Results: Adolecents (53.1% male) had a mean age of 169 ± 32 months. The frequency of AA, AG, and GG genotypes were 78.6%, 19.1%, and 2.3%, respectively. Hepatosteatosis was present in 46.9% among obese group. The obese group had more AG/GG genotypes ($p = 0.038$), however, genotypes were independent from clinical and laboratory features with anecdotal evidence (total bilirubin, TSH, triglyceride, birth weight, systolic blood pressure) and moderate evidence (BMI-SDS, WC-SDS, diastolic blood pressure). Also, hepatosteatosis was independent from LCP1 gene among obese group (BF_{10}:0.316) and among all cases (BF_{10}:0.171) with moderate evidence.

Conclusion: GG and AG genotypes were more frequent among obese group; however, these variants were independent from blood pressure, BMI, laboratory, and especially hepatosteatosis with anecdotal and moderate evidence.

Table 1. LCP1 rs7324845 Gene Variant distribution among groups

AA vs G	Control n(%)	Obese n(%)	Total	p
AA	40 (88.9)	63 (73.3)	103	0,038
AG / GG	5 (11.1)	23 (26.7)	28	
Total	45	86	131	

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P05.062.C Novel variant in the activin A receptor like type 1 *ACVRL1* gene associated with hereditary hemorrhagic telangiectasia

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Background/objectives: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder characterized by recurrent epistaxis, skin/mucocutaneous telangiectasia, and organ/visceral arteriovenous malformations. Mutations in the *ENG*, *ACVRL1*, and *SMAD4* genes mainly cause HHT. *ACVRL1* is a type I receptor for TGF-beta family members and is important in vascular development.

Patients and methods: A 14-year-old female was clinically diagnosed with HHT. Saliva samples were obtained from the patient and her family members, followed by genomic DNA extraction. The proband's HHT-related genes were sequenced using an Illumina platform. Then, Sanger sequencing was employed to confirm the variant of *ACVRL1* detected in other family members with HHT.

Results: Genomic analysis revealed a novel missense variant in the extracellular domain coding region of the *ACVRL1* gene. No other genetic abnormalities were detected. Then, we confirmed that the family members with HHT shared the variant. Biochemical analyses demonstrated that the variant gene product impaired the function of the ALK1 receptor, suggesting that the amino acid substitution affects BMP ligands' binding to the receptor. In silico structural analysis further supported the pathogenic nature of the variant.

Conclusion: Identifying this novel missense mutation in the *ACVRL1* gene corroborated the patient's clinical diagnosis of HHT. We revealed that the mutation results in a loss of function of the *ACVRL1*, likely contributing to the development of HHT. These findings suggest that this variant is a causative mutation for HHT, expanding the mutation spectrum of the *ACVRL1* gene and providing insights into its molecular pathology.

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P05.063.D Investigation of new genetic variants by whole exome Sequencing (WES) in Turkish MODY-X families

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Background: Maturity-onset diabetes of the young (MODY) is the most common type of monogenic diabetes. Different subtypes associated with 14 different genes have been described. For genetic diagnosis of MODY, NGS based multigene targeted panels for known genes are commonly applied. The genetic etiology, however; remains unexplained in approximately 20–30% of patients (MODY-X). In this study, we aimed to identify additional genes and novel variants in the MODY-X families.

Material and methods: WES was performed in at least two related patients in 5 families ($n = 10$) who were admitted to our clinic between 2020 and 2023 with a prediagnosis of MODY and whose genetic diagnosis could not be established by multigene targeted panels for the etiology of MODY.

Results: Two novel variants in *GIGYF1* gene in 2 different families and 1 novel variant in *SLC30A8* gene in 3 individuals from 2 different families were detected. Individual family analysis showed variants considered to be significant in previously reported genes of monogenic diabetes. In addition, novel variants in *GCKR*, *RFX7*, *RAP1GAP*, *EXOC7* genes, which were not previously shown in diabetic patients were identified.

Conclusion: WES contributes to deepen genetic research, identify new genes and variants and better understand the molecular pathogenesis of this disease. In addition to genes previously associated with diabetes, this study identified new candidate genes that may be associated with MODY. This is the first WES study of this scale in the MODY-X group in the Turkish population.

C.O.I. Disclosure:
M. Ozen: None. **B. Kesriklioglu:** None.

P05.065.B Investigation of absolute mtdna copy number and gene variants associated with mtdna function in obese adolescents

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Background: Obesity in adolescents is the most challenging public health problem of the twenty-first century and is associated with increased morbidity and premature death. This study aimed to investigate the absolute mtDNA copy number and peroxisome proliferator-activated receptor family (PPARs) and Peroxisome proliferator-activated receptor-γ coactivator-1-α (PPARGC1A) gene variants in obese adolescents.

Material and methods: A total of 183 individuals, 121 adolescents diagnosed with obesity and 62 healthy adolescents as the control group, were included in the study. PPARA, PPARG, PPARD and PPARGC1A gene variants in DNA samples isolated from the peripheral blood of the individuals were analyzed quantitatively using the PCR-RFLP method, and absolute mtDNA copy number was analyzed quantitatively using real-time PCR.

Results: When obese adolescents and healthy controls were compared, no statistically significant relationship was found in mtDNA copy number. No significant difference was found in genotype and allele frequencies of PPARA rs1800206, PPARG rs1801282, PPARD rs2016520 and PPARGC1A rs8192678 gene variants. However, PPARGC1A rs8192678 variant GG genotype was found significantly higher in obese adolescents and GA genotype was found significantly higher in healthy controls ($p = 0.033$, $p = 0.041$, respectively).

Conclusion: This is the first study in the literature to investigate absolute mtDNA copy number and PPARs and PPARGCA1 gene variants in obese adolescents. It has been shown that the high frequency of PPARGC1A-rs8192678 missense mutation GG genotype may be associated with adolescent obesity. However, larger study groups and evaluation of clinical data in patient groups of different ethnic origins will provide better clarification of this relationship.

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P05.066.C Variant in *RAF1* in a patient with 46,XY differences of sex development (DSD)

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Background: *RAF1* (Raf-1 proto-oncogene, serine/threonine kinase) encodes a kinase involved in the MAPK/ERK signaling pathway, crucial for cell growth, differentiation, and survival. Activating pathogenic variants in *RAF1* often result in hyperactivation of signaling pathways, leading to developmental abnormalities or uncontrolled cell proliferation and are associated with Rasopathies, like Noonan syndrome (OMIM #611553) as well as Noonan syndrome with multiple lentigines (OMIM #611554). Clinical features of Noonan syndrome with multiple lentigines include multiple lentigines typically from the age four, café-au-lait spots, craniofacial dysmorphisms, cardiovascular anomalies, sensorineural hearing loss, short stature, and genital anomalies.

Material and methods: We acquired the patient's family history and clinical as well as hormonal data. Chromosome banding analysis and exome sequencing were performed using blood samples. The disease's severity of our patient was assessed and literature search for similar patients was performed.

Results: The patient was born following in vitro fertilization to non-consanguineous parents. The family history was uneventful. He presented with penoscrotal hypospadias, multiple Café-au-lait spots, pronounced axillary freckling and proximal placement of the thumbs. Chromosome banding analysis showed a 46,XY karyotype. Whole-exome sequencing revealed a heterozygous variant of unknown significance in *RAF1* (NM_002880.4:c.1687C>G; p.Arg563Gly). The variant was not present in the gnomAD database and was located in a conserved protein domain involved in protein-protein interaction (CADD score: 28). A segregation analysis is pending.

Conclusion: We report a patient with 46,XY DSD carrying a variant in RAF1. So far only a few patients with pathogenic variants in *RAF1* and a DSD phenotype have been described.

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N. Hornig: None. **I. Nagel:** None. **M. Salewski:** None. **O. Hiort:** None. **A. Caliebe:** None. **M. Spielmann:** None.

P05.067.D The Israeli Wolfram Syndrome registry: a unique phenotypic landscape

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Introduction: Wolfram Syndrome 1 (WFS1) is characterized by diabetes insipidus, diabetes mellitus, optic atrophy (OA), deafness, along with additional multisystem manifestations. Wolfram-like syndrome (WLS) is heralded by hearing impairment sharing many WFS1 manifestations. Both conditions are caused by pathogenic *WFS1* variations, inherited in an autosomal recessive (WFS1) and autosomal dominant (WLS) manner. Prevalence of WFS1 spectrum disorders varies worldwide, while a founder variant: c.1672C>T, p.(Arg558Cys), present in 1.34% of Ashkenazim, leads to high local prevalence with unique manifestations. We describe herein the national Israeli registry of WFS1 spectrum disorders.

Materials and methods: Individuals attending the WFS1 designated clinic at Sheba Medical Center were enrolled, along with individuals referred from centers nationwide. Ongoing clinical data, based on a designated questionnaire was updated at regular intervals.

Results: Since 2021, 32 individuals (10 males, age range 4–75 years), were enrolled (WFS1:$n = 28$, WLS $= 4$), representing 22 families. Homozygotes ($n = 22$) or compound heterozygotes ($n = 4$) of the c.1672C>T founder variant, were significantly older at diagnosis, with delayed OA compared to individuals harboring other variants ($n = 6$, $p < 0.01$). Among the former, females were diagnosed at a significantly older age ($n = 14$) ($p < 0.01$). Hearing loss was present among all WLS ($n = 3$), and least common among homozygotes for the c.1672C>T variant ($n = 4$, 18%). Additional symptoms included urinary ($n = 18$), psychiatric ($n = 6$), impaired balance ($n = 5$), cognitive decline ($n = 3$) among others.

Conclusions: The Israeli registry represents the unique national landscape of WFS1 spectrum disorders. This registry may enhance

our understanding, improve patient care, and serve as an infrastructure for clinical trials and drug development.

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P05.068.A Novel biallelic Splicing variant identified in PKHD1 gene in a consanguineous Iranian pedigree with autosomal recessive polycystic kidney disease

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Background: Variants of the polycystic kidney and hepatic disease 1 (PKHD1) gene are associated with polycystic kidney disease 4, with or without hepatic disease (PKD4). The most typical disease expression occurs in neonates and includes a history of oligohydramnios, massively enlarged kidneys, and the 'Potter' sequence with pulmonary hypoplasia that leads to respiratory insufficiency and perinatal death in approximately 30% of affected newborns. This study aimed to identify the genetic cause in an Iranian consanguineous pedigree with apparently autosomal recessive polycystic kidney disease (ARPKD).

Material and methods: The genomic DNA was extracted from the affected fetal tissue. Whole-exome sequencing (WES) and bioinformatic analysis were used to identify the potential genetic cause. The identified variant in PKHD1 gene was confirmed in the fetus and peripheral blood samples of the parents bysanger sequencing.

Results: Novel biallelic variant, chr6:51890981 T>G, was identified in the PKHD1 gene (NM_138694.4, c.3629-2A>C) by WES; that has not been previously reported. Based on the analysis performed on the data obtained from sanger sequencing, the couple were carriers. Bioinformatic splice prediction tools revealed that the variant strongly impacts splice site function. Based on the ACMG guidelines, this variant is categorized as likely pathogenic.

Conclusion: A novel likely pathogenic variant of PKHD1, chr6:51890981 T>G, was found in an Iranian consanguineous pedigree with ARPKD, which enriches the variant spectrum of the PKHD1 gene and provides a basis for genetic counseling and the diagnosis of ARPKD.

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R. Ebrahimi Askari: None. **S. Sadeghzadeh:** None. **N. Ghasemi:** None.

P05.069.B A mutation in exon 1 of the androgen receptor gene results in complete androgen insensitivity syndrome

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Background: Androgen insensitivity syndrome is an X-linked recessive disease due to loss-of-function mutations of the *AR* gene (Xq 11–12). The disease affects individuals phenotypically females with 46, XY karyotype. Different *AR* mutations lead to diverse impairment of protein function, thus determining a wide spectrum of phenotypes: mild, partial, complete (CAIS) insensitivity to androgens (OMIM#300068). Currently, about 1000 mutations have been identified in *AR* and linked to AIS, mainly located in the hormone-binding domain.

Material and methods: We describe a female with CAIS, diagnosed at 18 years old due to primary amenorrhea, with 46, XY karyotype and absence of Müllerian structures. Gonadectomy was performed, confirming testicular tissue without signs of malignancy. We performed a clinical exome using next-generation sequencing (SureSelect CDCFE, Agilent-Technologies), using Illumina Platform.

Results: The proband showed a small insertion in exon 1 of the *AR*: (NM_000044.6): c.195_196insTAGCAGCAG, p.Gln65_Gln66insTerGlnGln. The variant has never been described; it was classified as likely pathogenic in databases (e.g. Varsome). Indeed, this variant introduces a stop codon, likely leading to a premature protein truncation, and consequent loss of function.

Conclusion: We identified a small insertion within exon 1 of *AR* gene, near to the start codon. To our knowledge, a similar genetic anomaly was only described once in a proband with clinical manifestations similar to the present case (https://doi.org/10.1016/j.steroids.2019.02.007). Our findings reinforce the role of exon 1 insertions in determining AIS and further expand the database of *AR* gene mutations.

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P05.070.C An atypical case of Alström Syndrome: challenging the Classic phenotype

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Background: Alström syndrome (AS, MIM#203800) is an autosomal recessive disorder caused by biallelic pathogenic variants in the ALMS1 gene. AS is characterized by rod-cone dystrophy, sensorineural hearing loss (SNHL), obesity, cardiomyopathy, non-alcoholic fatty liver disease and chronic renal disease. Affected individuals may also exhibit developmental delay and endocrine problems including insulin resistance, type 2 diabetes mellitus, hypertriglyceridemia, hypothyroidism, hypogonadism and obesity. Although there is wide clinical variability among affected individuals, even within the same family, rod-cone dystrophy, obesity, short stature, insulin resistance, and SNHL are typical findings observed in most patients, aiding in the prediagnosis of the disease. Here, we present a male patient whose body weight was below −2 SD, in contrast to the commonly observed obesity in patients with AS.

Material and methods: Exome sequencing was performed.

Results: The patient presented with microcephaly, failure to thrive (BMI: −1.44 SD), dysmorphic features, bilateral SNHL, astigmatism, hypothyroidism, adrenal insufficiency, intellectual disability, and a history of acute rheumatic fever carditis. Abdominal ultrasound, liver function tests, and renal function

tests were normal. Karyotype analysis and chromosomal microarray were normal. Exome sequencing identified a novel, de novo homozygous variant c.1223_1237+1del in *ALMS1*, classified as "likely pathogenic" according to ACMG criteria.

Conclusion: Although AS presents with a broad spectrum of clinical findings, obesity is the most common feature after rod-cone dystrophy across all age groups. However, it is important to recognize that AS can also occur in non-obese individuals who exhibit other characteristic findings.

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P06 Skeletal, connective tissue, ectodermal and skin disorders

P06.001.D Cellular senescence in pseudoxanthoma elasticum

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Background: Pseudoxanthoma elasticum (PXE) is a rare hereditary disorder caused by bi-allelic *ABCC6* mutations, leading to pathological calcification in the skin, arteries, and eyes. Increasing evidence suggests that cellular senescence—a state of irreversible cell cycle arrest triggered by DNA damage, oxidative stress, and telomere shortening—plays a key role in PXE pathophysiology. Senescent cells contribute to tissue dysfunction through the senescence-associated secretory phenotype (SASP), which promotes chronic inflammation and extracellular matrix (ECM) remodeling. NF-κB, a central regulator of SASP, accelerates senescence, while IL-6 amplifies pro-inflammatory signaling and exacerbates disease progression. Furthermore, excessive activation of the DNA damage response (DDR) may further drive pathological changes in PXE. This study investigates the roles of NF-κB, IL-6, and DDR activation in PXE to identify potential therapeutic targets.

Material and methods: Skin tissue (n = 4) and dermal fibroblasts (n = 9) from PXE patients and matched controls (n = 9) were analyzed. NF-κB was assessed via immunofluorescence, and calcium deposits were visualized using Von Kossa staining. RT-qPCR was used to evaluate differential gene expression of *NF-κB*, *IL6*, DDR targets like *PARP1* and downstream targets.

Results: NF-κB was highly expressed in PXE skin, co-localizing with osteogenic markers and calcium deposits. PXE fibroblasts showed increased *NF-κB*, *PIK3CA*, *AKT1*, *PARP1*, *ATM*, *p21*, *p53*, *RUNX2* and *IL6* mRNA levels, while *SIRT1* mRNA levels were decreased.

Conclusion: Disrupted pathways in PXE, including DNA damage response, PARP1 activation, and NF-κB upregulation, link PXE to cellular senescence. Targeting senescence pathways and SASP may provide new therapeutic strategies to slow disease progression and improve patient outcomes.

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P06.002.A Body site-specific transcriptomic signature in psoriatic lesions

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Background: Non-invasive methods like tape-stripping have been increasingly used to identify response biomarkers in psoriasis. Some psoriatic lesions show resistance to biological therapies and respond differently. This study aimed to investigate molecular differences between individual psoriatic lesions.

Material and methods: Tape-stripping method was used to obtain skin samples from active lesions. RNA from psoriatic skin was extracted of 24 adult Slovenian patients with plaque psoriasis. Skin samples were obtained from four different body parts (trunk, scalp, upper, and lower extremities), depending on the patient's clinical presentation. All patients were naïve to biological therapy. Additionally, both lncRNA and mRNA 100 bp paired-end libraries were constructed. Furthermore, RNA-seq analysis was performed to compare different anatomical sites, followed by gene ontology analysis.

Results: RNA-seq analysis revealed distinct miRNA expression patterns across different anatomical psoriatic lesions. Our results highlight several miRNAs that may play pivotal roles in psoriasis pathogenesis and lesion-specific molecular differences. Notably, miR-4323, miR-203a, miR-4274, *MIRLET7A1*, *MIRLET7G*, miR-205, and miR-200b were identified across multiple comparisons, suggesting their involvement in the regulation of psoriasis lesions. These miRNAs show potential as key regulators of the molecular divergence between different lesion types; however, their exact roles in the disease's pathophysiology remain to be fully elucidated.

Conclusion: The present study highlights the importance of our results in elucidating molecular pathways in different anatomical psoriatic lesions. These findings provide valuable insights into psoriatic lesion heterogeneity and could aid in identifying novel biomarkers and therapeutic targets for difficult-to-treat psoriasis cases.

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P06.003.B Homozygous *IGF1* receptor (*IGF1R*) in-frame deletion variant associated with microcephalic dwarfism and impaired bone maturation

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Background: IGF1R, a ubiquitous cell-surface receptor belonging to the insulin receptor (INSR) sub-family of receptor tyrosine kinases, mediates IGF-I's growth-promoting actions. Most pathogenic *IGF1R* variants are heterozygous loss-of-function mutations causing haploinsufficiency and moderate growth impairment. Very rare biallelic hypomorphic variants lead to severe growth impairment and microcephaly. To date, only 10 patients with homozygous or compound heterozygous *IGF1R* variants have been reported. Here we report a novel homozygous *IGF1R* pathogenic variant in two siblings with microcephalic dwarfism and additional comorbidities, demonstrating its pathogenicity through various functional studies.

Material and Methods: Whole-exome sequencing was performed in two siblings presenting with microcephalic dwarfism, mild intellectual disability, deafness, congenital cardiac malformations, partial vermis hypoplasia, dysmorphic features, and skeletal abnormalities. Functional studies included patient-derived fibroblasts analysis, 3D protein modeling, generation of a CRISPR/Cas9 gene-edited $Igf1r^{KI/KI}$ knock-in mouse model, and patient-based iPSC models.

Results: A homozygous *IGF1R* c.2132_2143del variant was identified. This in-frame deletion in the extracellular region did not reduce receptor expression or alter cell surface localization but significantly compromised IGF-I-induced signaling. The $Igf1r^{KI/KI}$ mouse model recapitulated severe growth impairment, IGF-I resistance, and impaired skeletal integrity, including reduced bone strength and defective anterior fontanel calcification. Patient-derived iPSCs showed reduced bone mineralization, partly due to RUNX2 dysregulation, which was reversed upon gene-editing to wild-type.

Conclusion: This study report a rare novel homozygous hypomorphic *IGF1R* variant and highlights the critical importance of IGF1R in osteogenesis and skeletal maturation through analyses of patient-derived fibroblasts, the first viable knock-in mouse model, and human iPSC models.

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P06.004.C Zebrafish as a model for Myhre syndrome: growth deficits and vascular narrowing (Award Candidate)

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Background: Myhre syndrome (MS, MIM 139210) is a rare multisystemic disorder caused by recurrent pathogenic missense variants in SMAD4. The clinical features comprise variable neurocognitive development, autism, recognizable craniofacial features, short stature, hearing loss, a thickened skin, joint limitations, diverse cardiovascular and airway manifestations including arterial stenosis, and increased fibrosis often following trauma or surgery.

Material and methods: We developed a zebrafish model for Myhre syndrome. Using CRISPR technology, we generated heterozygous and homozygous smad4a p.Ile495Thr knock-in (KI) zebrafish, which correspond to the known human SMAD4 p.Ile500Thr variant. The knock-in was crossed with the transgenic Tg(kdrl:EGFP) zebrafish line to visualize the ventral aorta and aortic branches.

Results: At 5 days post-fertilization (dpf), vasculogenesis was not different in p.Ile495Thr KI versus wild type controls. At 10 dpf, heterozygous and homozygous KI zebrafish exhibited a significant reduced diameter of the ventral aorta between aortic arch two and three, as well as a decreased aorta diameter adjacent to the bulbus arteriosus compared to wild-type controls. Cardiac ultrasounds of 6-month-old zebrafish showed no significant abnormalities. Measurements of 4-month-old zebrafish revealed decreased body length in homozygous mutants compared to wild type controls. Mortality at three months post fertilization seems higher in homozygous KI zebrafish (up to 50%).

Conclusion: Our zebrafish model for MS exhibits reduced body length, narrowing of the ventral aorta, and potentially increased mortality. Further phenotypic assessment and multi-omic analyses are underway to elucidate the underlying disease mechanisms.

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P06.005.D Genome sequencing in a cohort of 32 fetuses with genetic skeletal disorders

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Background: Approximately 200 genetic skeletal disorders can present prenatally, detectable through ultrasound abnormalities during pregnancy. Severe forms are typically identified during the first or second trimester, whereas milder phenotypes are recognized later, in the third trimester. Diagnosing skeletal dysplasia prenatally is challenging due to the large number of disorders and the frequent overlap in clinical findings that can be detected by ultrasound.

Material and methods: This study, conducted at Karolinska University Hospital between 2015 and 2022, examines the genetic and radiographic findings in 32 fetuses (14 female and 18 male, from unrelated families) with skeletal abnormalities detected on prenatal ultrasound and confirmed by radiographs either at birth or after pregnancy termination. Fetal DNA samples underwent genome sequencing using an in silico skeletal dysplasia gene panel as singletons for all fetuses. As a second step, trio genome sequencing was performed for six fetuses with unsolved molecular diagnoses, involving both the fetus and parents.

Results: The diagnostic yield of genome sequencing was 72%, with pathogenic or likely pathogenic variants identified in 23 of the 32 fetuses. Additionally, four variants of uncertain significance, strongly suspected to be causative based on clinical and radiographic features, as well as structural protein analyses, were identified in four fetuses with autosomal recessive conditions. The molecular diagnoses for five fetuses remain unsolved.

Conclusion: By combining detailed phenotypic data with singleton genome sequencing, we achieved a genetic diagnosis in 72% of the 32 cases of fetal genetic skeletal disorders investigated at the Karolinska University Hospital.

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P06.006.A Functional and clinical significance of splicing variants in patients with osteogenesis imperfecta

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Research Centre for Medical Genetics, Moscow, Russian Federation.

Background: Osteogenesis imperfecta types I-IV (OI) represents a group of inherited bone disorders. Splicing variants constitute a significant proportion of all pathogenic variants, yet they remain among the most challenging to interpret due to their diverse mechanisms of altering mRNA structure and function.

Material and methods: For molecular diagnostics of patients, WES or WGS was performed. SpliceAI tool was used to predict splicing variants. The SpliceAI tool was utilized to predict the impact of splicing variants. To investigate splicing alterations, we constructed 12 minigenes encompassing all exons of *COL1A1* and 7 minigenes containing 20 out of 52 exons of *COL1A2*. To assess splicing patterns, HEK293T cells were transfected with variant-containing vectors, and splicing alterations were analyzed using RT-PCR, Sanger sequencing, and fragment analysis.

Results: We collected a cohort of 184 patients with various types of osteogenesis imperfecta (OI). Among them, 133 harbored variants in COL1A1, and 51 had variants in COL1A2. Bioinformatic analysis identified 29 variants in COL1A1 and 9 variants in COL1A2 as potentially affecting splicing. Additionally, we selected 40 variants from the literature, including 12 exonic variants, 19 lethal variants, and 13 splice site region deletions. We also included 10 frequent variants from gnomAD with high-confidence predictions. Overall, 68 of 71 variants showed splicing changes, including exon skipping causing in-frame deletions or out-of-frame shifts, leading to frameshifts and premature stop codons.

Conclusion: In total, we analyzed 71 splicing variants in patients with OI, revealing how diverse splicing alterations correlate with the severity of clinical presentations.

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P06.007.B Knock-in mice harbouring an auriculocondylar syndrome-causing *PLCB4* variant display anomalies of the jaw skeleton and of neonatal breathing

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Background: Auriculocondylar syndrome (ACS) is a rare mandibulofacial dysostosis characterized by "question mark" ear dysplasia and homeotic transformation of the lower jaw into an upper jaw-like structure, probably due to deregulation of the endothelin pathway during development of the branchial arches. Some ACS patients display hypoventilation during sleep. Heterozygous missense variants clustered in the catalytic domain of *PLCB4* are the most frequent cause of ACS. Here we analysed the developmental consequences of an ACS-causing variant in *Plcb4* knock-in mice.

Material and methods: Mice harbouring the recurrent *Plcb4* variant p.Arg621Cys (R621C) were generated by targeted replacement. Craniofacial structures were examined at embryonic day 18.5 by skeletal staining. Respiratory flow and abdominal movements were measured for one hour at birth. Obstructive breaths were revealed by abdominal movements during apnoea.

Results: $Plcb4^{R621C/R621C}$ mice have a shorter mandible and hypoplasia of the mandibular processes, as observed in other mouse models with reduced endothelin signalling in the mandibular prominence. $Plcb4^{R621C/R621C}$ and $Plcb4^{R621C/+}$ mice have an ectopic bone on either side of the basisphenoid that variably extends from the pterygoid process to the tympanic ring. Homozygous pups die within 12 hours of birth due to severe hypoventilation, apnoeas and obstructive breaths, accompanied by loss of chemosensitivity to hypercapnia. Heterozygotes show normal ventilation in air and a reduced chemosensitivity to hypercapnia that normalizes by 8 days of life.

Conclusion: Our data suggest a negative effect of the *Plcb4* variant on endothelin signalling in the mandibular prominence and an important role for *Plcb4* in the control of breathing.

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P06.008.C Unraveling the complex NOTCH1-SERCA2 interplay in Darier's Disease: insights into genotype-phenotype correlations and pathogenic mechanisms

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Ambrosini[2], Davide Martorana[2], Patrizia Caggiati[2], Bruno Lorusso[1], Vera Uliana[2], Giovanni Roti[1], Antonio Percesepe[1]

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Background: Darier's Disease(DD) is a rare dominantly inherited genodermatosis caused by *ATP2A2* variants, leading to loss of desmosomal adhesion and abnormal keratinization. *ATP2A2* encodes the SERCA2 transporter for calcium trafficking to the endoplasmic reticulum. The molecular mechanism underneath *ATP2A2* alterations is not understood. Interestingly, in other disease models, SERCA2 regulates NOTCH1 signaling, and its inhibition inactivates the pathway.

Material and methods: we collected patients' blood and skin biopsies: i)to perform transcriptomic RNA-sequencing analysis and Spatial transcriptomics on affected and unaffected subjects; ii)to generate keratinocytes primary cells for expression analysis of the NOTCH1-downstream effector targets(*HES1*,*HEY1*,*c-MYC*); iii)to investigate NOTCH1 signaling deregulations through immunohistochemistry on FFPE bioptic samples. Furthermore, we generated variant specific-*ATP2A2* overexpression plasmid library to evaluate variant impact on the NOTCH1 signaling by performing in vitro functional and biochemical assays(Western-blot,qPCR,Luciferase-assays).

Results: The project successfully identified a pathogenic role of NOTCH1 receptor-mediated pathway deregulations which correlate with the genetic and the histological features observed in patients with Darier's disease. Different variants were found to have distinct effects on NOTCH1 signaling, highlighting different impacts on disease manifestations. Moreover, we define a DD transcriptomic gene signature and spatial profiles, focused on metabolic, ribosomal and immunological deregulations. We pointed out a molecular dependency of NOTCH1 pathways and *ATP2A2* defects through the effects evaluation of patient-specific variant overexpression and by ex vivo assays based on patient-derived primary cell lines and tissue IHC.

Conclusion: Gaining insight into the molecular network between SERCA2 and NOTCH1 in DD could pave the way for the development of novel and effective therapeutic approaches.

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P06.009.D A novel form of autosomal dominant spondylocostal dysostosis in three unrelated families caused by the same heterozygous pathogenic variant in *MESP2*

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Background: Spondylocostal dysostosis (SCD) is a rare genetic bone disorder characterized by disproportionate short stature, scoliosis, and defects of vertebrae and rib formation occurring during embryonic development. SCD typically shows a recessive inheritance and several causal genes have been identified. These genes play a crucial role in somitogenesis and the subsequent formation of the vertebral column. Only in one family with a dominant inheritance a heterozygous mutation in TBX6 was identified.

Material and methods: Genome sequencing was performed on three TBX6-negative unrelated families with dominant SCD. Prediction programs were used to assess the impact of the identified mutation. The effect of the mutation on Notch signaling and subcellular protein localization was verified by immunostaining and luciferase reporter assays.

Results: Genome sequencing on a Belgian family with dominant SCD led to the identification of a heterozygous pathogenic MESP2 variant (c.268G>A; p.Glu90Lys). The same mutation was identified in a Brazilian and a Spanish family with a dominant form of SCD. The p.Glu90Lys variant was evaluated as disease-causing in silico and affects a highly-conserved amino acid in the bHLH domain of the MESP2 transcription factor, responsible for DNA binding. Functional studies demonstrated an impaired regulation of LFNG expression and a more dominant nuclear localization of p.Glu90Lys MESP2. These data strongly suggest a dominant negative effect.

Conclusion: We provide the first evidence that a heterozygous mutation in MESP2 can cause a dominant form of SCD. Ongoing studies aim to further determine the effects of this mutation on somitogenesis and vertebral segmentation.

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P06.010.A Familial hidradenitis suppurativa linked to GJB3 mutation: disruptions in keratinocyte proliferation and differentiation

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Background: Mutations in key genes can profoundly impact skin health, altering fundamental biological processes and contributing to the development of chronic conditions. Among these, familial hidradenitis suppurativa (fHS) remains a particularly challenging disorder, with its complex genetic and environmental interactions still not fully understood.

Material and methods: To explore potential genetic contributions to fHS we performed WES on DNA from multiple affected members, looking for new or rare heterozygous variants with a significant predicted impact on protein function. To investigate the functional impact, we introduced the SNV of interest into the keratinocyte cell line using CRISPR/Cas9 genome editing, generated a 3D human epidermal equivalents and performed RT-PCR, western-blot, microscopy assays, cell-proliferation assays and proteomic analysis.

Results: WES analysis revealed a shared heterozygous frameshift SNV in the GJB3 gene, the first instance of a GJB3 mutation being linked to fHS. The GJB3 gene encodes a connexin protein, which plays a crucial role in gap junctions, the structures

responsible for enabling communication between keratinocytes. This mutation appears to significantly change GJB3 gene and protein expression, alter the gap junction morphology and significantly increase the proliferation rate. Gene ontology enrichment analysis of proteomic data highlighted significant alterations in molecular functions and biological processes related to cell adhesion, skin structure, and epidermal maintenance.

Conclusion: This study provides the first evidence linking a variation in GJB3 gene to fHS, opening new avenues for understanding the molecular mechanisms of the disease. Our functional investigation revealed that variation compromises cell-cell communication channels, potentially leading to dysregulated keratinocyte proliferation and impaired differentiation.

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P06.011.B THBS2 gene variants as a putative emerging molecular cause for hypermobile Ehlers Danlos Syndrome/ Hypermobility spectrum disorders. A case series

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Background: The Ehlers Danlos syndromes (EDS) are a group of hereditary connective tissue disorders with common features including joint hypermobility, hyperextensible skin and tissue fragility. Genetic testing is informative for a definitive diagnosis in EDS subtypes with a known causative gene, but some patients with a clinical diagnosis, especially those with the hypermobile type (hEDS), still lack confirmation at molecular analyses. Moreover, clinical distinction between hEDS and Hypermobility Spectrum disordes (HSD) can be challenging. Recently, a novel dominantly inherited EDS-like phenotype has been described in association with a missense variant in the *THBS2* gene. Main reported features were joint dislocations, atrophic scarring, prolonged bleeding time and age-related aortic dilatation and rupture.

Material and methods: Negative whole exome sequencing analyses of 209 patients with a clinical diagnosis of hEDS or HSD were revaluated for variants in THBS2. Clinical features at first referral and those resulted from reverse phenotyping of the identified cases were collected and compared to those already reported.

Results: We identified 7 patients (3%) carrying a missense variant in *THBS2*. In silico predictions indicated a likely disruptive effect for each variant. Although our case series has not yet revealed major vascular complications, we did observe the presence of vascular malformations and/or aortic ectasia.

Conclusion: Given its recent identification, this new putative form of EDS necessitates further validation in larger cohorts. Considering the late onset of aortic rupture in the initial family described, our findings suggest that precautionary surveillance for vascular complications should be considered for patients harbouring *THBS2* variants.

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P06.012.C Further evidence that bi-allelic pathogenic variants in IFT74 are a novel cause of skeletal ciliopathy and implications for diagnostic testing

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Background: Skeletal ciliopathies are autosomal recessive disorders sharing characteristic clinical and radiographic features. Genes involved frequently encode intraflagellar transport proteins. Bi-allelic *IFT74* variants are rare causes of Bardet-Biedl and Joubert syndrome, and isolated spermatogenic failure (OMIM: 608040). Bakey et al. recently showed that putative truncating variants in *IFT74* can also present with a predominant skeletal phenotype.

Methods: Trio exome sequencing (ES) followed by analysis of a filtered skeletal dysplasia gene panel was performed. RNA-sequencing was done on patients' skin fibroblasts to detect pathogenic expression outliers for a ciliopathy gene panel. Confirmation of RNA- sequencing result at the DNA- level was achieved using Multiplex Amplicon Quantification (MAQ).

Results: A male fetus presented at 22 weeks with ultrasonographic findings of a skeletal ciliopathy. Parents were consanguineous. Postmortem evaluation confirmed long narrow thorax with short ribs, handlebar clavicles, brachydactyly and trident acetabula. ES revealed no variants of interest in *IFT80* or *KIAA0586*, located in ROHs. Only a mono-allelic pathogenic variant in *IFT81* was detected. Subsequent RNA-sequencing was performed and revealed absent expression of *IFT74* exon 2. Re-analysis of exome data showed no coverage for this region and MAQ confirmed a homozygous pathogenic exon 2 deletion.

Conclusion: Our results further establish the role of *IFT74* as a novel cause of skeletal ciliopathy, most resembling the short-rib thoracic dysplasia subtype (formerly asphyxiating thoracic dysplasia). Exon 2 deletions are recurrent and may be readily missed on routine ES. Diagnostic work-up for skeletal ciliopathies should include *IFT74* sequencing and targeted CNV- analysis.

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P06.013.D Arylsulfatase L (ARSL) controls Glycosaminoglycan Sulfation during skeletal development

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Background: Proteoglycans, key components of the cartilage extracellular matrix (ECM), are decorated with glycosaminoglycan (GAG) chains whose sulfation patterns are tightly regulated during

biosynthesis. Aberrant GAG sulfation has been implicated in multiple skeletal disorders. Among them, X-linked chondrodysplasia punctata (CDPX) is a rare skeletal dysplasia characterized by abnormal cartilage development and impaired endochondral ossification. The disease is caused by mutations in the ARSL gene, encoding arylsulfatase L, a unique Golgi-localized enzyme. To date, however ARSL role in skeletal development remains unknown.

Material and methods: We combined in vitro biochemical assays, bioinformatic analyses, and unbiased LC-MS (Liquid chromatography - Mass Spectrometry) to identify ARSL substrates. Additionally, we developed a Medaka fish model lacking the *ARSL* ortholog (*Ol-Arsd*) to investigate the in vivo effects of ARSL deficiency through biochemical and morphological analyses, including μCT imaging.

Results: We demonstrate that ARSL desulfates chondroitin sulfate (CS) specifically at the 4-O-position, establishing CS as a bona fide substrate of ARSL. Loss of *Ol-Arsd* in Medaka fish resulted in hypersulfated CS at the 4-O-position, leading to defective perichondral and intramembranous ossification and skeletal abnormalities that mimic those observed in CDPX patients, including nasal hypoplasia and shortened skeletal elements

Conclusion: Our findings identify ARSL as a regulator of GAG desulfation in the Golgi and suggest a direct link between proteoglycan sulfation and CDPX manifestations.

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P06.014.A Diagnostic challenges in Neurofibromatosis type 1: an Italian cohort study between parental testing and cell line models generation

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Background: Neurofibromatosis type 1 (NF1) presentation is highly variable, and overlapping signs to other Rasopathies make differential diagnosis challenging, especially in incomplete phenotypes. We aimed to define the diagnostic rate of genetic analysis in NF1 patients and its improvement by parental testing and functional studies.

Material and methods: We reviewed data from 230 probands who underwent Rasopathies NGS panel and *NF1* MLPA testing for NF1 differential diagnosis between 2018 and 2024.

Results: 126 probands (55%) harboured *NF1* pathogenic/likely pathogenic (LP) variants, including 15 cases (6.5%) whose variants were upgraded from uncertain significance (VoUS) to LP due to their de novo nature. The disease spectrum ranged from mild to severe, with truncating/splicing variants enriched in the latter (68% vs 87%; $p = 0.007$). 31 patients (25%) did not fulfil the revised NF1 criteria (mean age:10.6y vs 18y for clinical diagnosis; $p = 0.02$). 12 probands (5.2%) displayed *NF1* VoUS, whereas alternative diagnoses were possible in 11 (4.7%) cases (5 *SPRED1*, 4 *LZTR1*, 2 *PTPN11*). For VoUS clarification, *NF1* cell models are critical when biopsies of affected tissues are unavailable. We demonstrated an EGF-inducible RAS pathway in immortalized non neoplastic hTERT RPE-1 and N-thy ori3-1 cells (up to 3-fold increase in RAS-GTP signal; $p = 0.002$) and used N-thy cells to generate NF1 H1348P mutant models via CRISPR/Cas9-mediated knock-in approach.

Conclusion: Our results widen the NF1 genotypic spectrum and underline the relevance of parental testing in the molecular diagnosis of incomplete phenotypes. We also defined a cell line suitable for VoUS functional studies, generating an NF1 mutant model.

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P06.015.B Multi-OMICs approach and computational drug repurposing: cellular pathogenesis and therapeutic perspectives for vascular Ehlers-Danlos syndrome

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Background: Vascular Ehlers-Danlos syndrome (vEDS) is a rare connective tissue disorder caused by heterozygous deleterious variants in COL3A1. Affected individuals have an increased risk of early death due to rupture of arteries, large intestine, and gravid uterus. The pathogenesis of vEDS is only partially understood and the disease remains without treatment. Three-dimensional (3D) spheroids represent an innovative cell culture model mimicking connective tissue-like properties to more effectively replicate of the in vivo cellular environment.

Material and methods: Transcriptomic, proteomic, ultrastructural analysis and high content imaging analysis on 2D fibroblast cultures and/or 3D spheroids from ten vEDS patients and four controls; computational drug repositioning on a set of FDA/EMA approved drugs.

Results: Transcriptomic analysis revealed upregulation of genes related to mitochondrial function, organellar ribosomal subunits and biosynthesis processes, indicating an enhanced adaptive metabolic response, whereas downregulation of genes involved in cell migration, differentiation and stress response highlighted abnormalities in cellular signalling and extracellular matrix maintenance. Proteomic analysis revealed that induced proteins were enriched for the mitochondrial matrix, minichromosome maintenance complex and biological processes involving low-density lipoprotein particles. Ultrastructural analysis and high

content imaging have documented an endoplasmic reticulum dilatation, increased autophagosomes and lipofuscin deposits. Computational drug repositioning analysis on a set of FDA/EMA drugs that are able to correct the defects associated to mutated COL3A1 is ongoing.

Conclusion: Our findings delineate a cellular pathogenesis for vEDS. The therapeutic potential of drugs identified as modulators of pathological cellular phenotypes will be evaluated in vitro in vEDS fibroblasts for future human application.

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P06.016.C SMAD7: a novel gene in Sclerosing bone dysplasia

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Background/objectives: Sclerosing bone dysplasia is a heterogeneous group of disorders caused by different genes that result in decreased bone resorption or, conversely, increased bone formation. Different signalling pathways involved in these pathologies can lead to an excessive increase of bone formation. The present case study concerns a patient with an atypical and generalised hyperostosis for whom targeted panel sequencing was negative. We used Whole Exome Sequencing (WES) and functional studies to find molecular basis of this disease.

Methods: WES, functional studies using primary fibroblast cells from the patient and controls (Western-Blot (WB); RNA-seq, RT- qPCR).

Results: This woman displays a severe facio-mandibular hyperostosis and diaphyseal sclerosis of the long bones. The first signs appeared in early childhood and worsened with age. WES revealed compound heterozygous missense variants in *SMAD7* gene, an inhibitor of SMADs pathways. Using primary fibroblasts, we found that the RNA and protein expressions of *SMAD7* were decreased in the patient cells compared with control cells. WB results showed that SMAD2/3 and p-SMAD3 levels were higher in mutated fibroblast cells than in control cells after TGF-β1 treatment. Furthermore, SMAD7-mutated cells showed increased RNA expression of ALPL, with reduced expression of RUNX2 and its target genes SP7 and COL1A1. Moreover, RANKL expression was abolished in SMAD7 mutated cells and OPG expression was slightly increased. RNAseq showed that genes involved in wnt and PI3K-AKT signalling pathways were upregulated.

Conclusion: Functional studies support the pathogenicity of SMAD7 variants in sclerosing bone dysplasia.

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P06.017.D Improving the diagnostic yield of skeletal dysplasia through targeted systematic re-analysis of known disease genes

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Background: Identifying the underlying genetic cause of a patient's rare disease is critical to provide appropriate counselling and access to novel treatments. Currently a prolonged diagnostic odyssey exists and often the underlying cause is never identified. Collectively 1 in 5000 individuals are affected by a skeletal dysplasia for which there are 552 known disease genes published in The Nosology of genetic skeletal disorders:2023 revision. More than 900 individuals with skeletal dysplasia were recruited for the 100,000 Genomes Project however at present only 27.3% (256/937) of the cases are listed as solved.

Material and methods: Utilising a targeted approach, systematic re-analysis of all genes present in Skeletal Nosology was completed for individuals enrolled in the 100,000 Genomes Project with an overarching diagnosis of skeletal dysplasia, currently listed as unsolved. Analysis was completed assessing both SNV and/or structural variants, respective of the disease genes inheritance pattern.

Results: Strong candidate variants in known disease genes were found for more than 3% of the currently listed as unsolved skeletal dysplasia cases. This included variants that for a myriad of reasons were not originally tiered at a level to be identified on original analysis including recent novel disease genes, splice site variants, and structural variants.

Conclusion: This project significantly improved the diagnostic yield of unsolved skeletal dysplasia cases and highlighted the diverse landscape of molecular mechanisms that underly skeletal dysplasia. It also demonstrated the potential for continued re-analysis of available data and the need for similar approaches to be utilised across other rare disease domains.

C.O.I. Disclosure:

M.F. Baxter: None.

P06.018.A Enhancing polygenic risk score accuracy for adolescent idiopathic scoliosis through an international GWAS meta-analysis

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Background: Adolescent idiopathic scoliosis (AIS) is a complex, three-dimensional spinal deformity affecting 2–3% of adolescents worldwide. Although genetic factors play a significant role in AIS susceptibility, the predictive accuracy of polygenic risk scores (PRS) remains limited. To improve PRS performance, we conducted an international meta-analysis of genome-wide association studies (GWASs) for AIS.

Materials and methods: We performed a meta-analysis of GWAS data from cohorts in Japan (5,427 cases and 155,983 controls), Hong Kong (977 cases and 2,085 controls), the US (1,503 cases and 20,153 controls), and Iceland (146 cases and 255,191 controls). Using these results, we inferred AIS-associated cell types

through heritability enrichment analysis and developed a polygenic risk score (PRS)-based prediction model.

Results: We identified 41 susceptibility loci, including 22 novel loci. The genetic background of AIS was strongly enriched in the regulatory regions of rhabdomyosarcoma, chondrocytes and myoblasts. By combining the regions with significant associations, 74.7% of AIS heritability could be explained by 9.8% of the genome. In a PRS analysis, we demonstrated that including more populations improved the AUC, a measure of predictability. Notably, the model prioritizing SNPs within significantly enriched regions achieved the highest AUC of 0.734 among all models.

Conclusion: We identified 22 novel AIS susceptibility loci by the meta-analysis. Three-quarters of AIS heritability could be attributed to the regulatory regions of specific musculoskeletal cell types. Our PRS-based prediction model incorporating global populations with these regions showed the highest predictability to date.

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P06.019.B Predictive factor of malignant tumoral development in multiple enchondromatosis

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Background: Ollier disease (OD) and Maffucci syndrome (MS) are extremely rare and complex disorders characterized by the abnormal development of multiple benign tumors in bones or soft tissues. These two disorders share identical clinical and histopathological features, leading to their classification under multiple enchondromatoses. Current knowledge on these pathologies is derived from sporadic case reports, genetic analyses of biopsies and retrospective studies. This accumulated knowledge higlighted a high risk of malignant transformation in patients, while the exact prevalence remains unknown. It is now demonstrated that somatic mutations in isocitrate dehydrogenase-1 (IDH1) or 2 (IDH2) are present in tumor tissues and hemangiomas

Methods: Observational cross-sectional study of 44 patients (37OD, 7MS)

Results: We found that the diagnosis remains radioclinical. Somatic mutations in IDH1 or IDH2 have been identified in affected tissues and rarely also in blood sample (4 patients). The prevalence of malignant tumoral lesions was 22% in OD patients, and 43% in MS patients. Multivariate analysis revealed that the localization of enchondromas in the axial skeleton, upper limbs, or when one side of the body was more affected than the other, was positively correlated with the development of malignant lesions.

Conclusion: Our study describes for the first time that patients with enchondromas localized in the axial skeleton, upper limbs, or with asymmetric body involvement have a higher risk of developing malignant lesions and should undergo more frequent follow-ups. Furthermore, the systematic search fro IDH1/IDH2 variant will be needed for future therapeutics including the possible use of IDH inhibitors.

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P06.020.C Whole genome association analysis in 450,000 individuals identifies rare and common variants associated with severe shoulder pathologies

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Background: Shoulder pathologies encompass a spectrum from soft tissue injuries to joint degeneration, impacting quality of life and often requiring surgery. Despite high prevalence, the genetic aetiology of shoulder pathologies remains under explored.

Material and methods: Using the UK Biobank (N = 448,436), we performed a whole-genome sequencing-based association analysis for five ICD10-defined shoulder pathologies: adhesive capsulitis, shoulder arthrosis, rotator cuff syndrome, shoulder calcific tendinitis, shoulder impingement, and three OPCS4-defined operations: shoulder replacement, subacromial decompression and rotator cuff repair. Genome-wide significant variants were followed up using a phenome-wide association study (PheWAS) including common disease, blood biomarkers, and plasma protein levels. FinnGen was used for replication where possible.

Results: We identified 2 common loci associated with severe shoulder conditions at $p < 1e\text{-}8$. We report a novel association with rs4326370 (MAF 22%) and shoulder replacement operation (OR 1.36 [1.30–1.43], $p = 4e\text{-}11$). The top two associations for this variant in the PheWAS were hip circumference ($p = 2e\text{-}7$) and shoulder arthrosis ($p = 4e\text{-}5$ in UKBB, $p = 0.003$ in the FinnGen replication). We replicated a well-known adhesive capsulitis association (rs62228066, $p = 8e\text{-}15$). We also found 3 novel rare variants for adhesive capsulitis (intronic in RBFOX3, intronic in ZNF516, and an intergenic variant on chromosome X) 2 for subacromial decompression (both intergenic on chromosomes 9 and 17) and 1 for shoulder replacement (intronic in PLEKHH2).

Conclusion: This is the first whole-genome sequencing-based analysis of these common shoulder pathologies. We confirm known genetic associations and report novel common and rare genetic loci. These findings improve our understanding of the aetiology of shoulder pathologies.

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P06.021.D Evaluation of mitochondrial DNA copy number and telomere length in patients with knee osteoarthritis in the Turkish population

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Background: Osteoarthritis (OA) is a degenerative joint disease that causes chronic joint pain and physical disability. Knee osteoarthritis (KOA) is a degenerative arthritis. Recent studies have demonstrated that mitochondrial DNA copy number (mtDNACN) is associated with OA and may contribute to its pathological development. Leukocyte telomere length (LTL) has been accepted as a clinical indicator of the risk of age-related diseases, such as OA. Therefore, we evaluated mtDNACN and LTL in Turkish patients with KOA.

Material and methods: A total of 228 subjects, including 147 patients with KOA and 81 healthy controls, participated in the study. The analyses were performed using quantitative real-time PCR (qPCR). The comparative Cq method was used to calculate the absolute $mtDNA_{CN}$ and LTL.

Results: Absolute $mtDNA_{CN}$ was increased in KOA patients compared with healthy controls ($p = 0.001$). The mean LTL was lower in patients with KOA than in controls ($p = 0.001$). Patients with $mtDNA_{CN}$> 286.8 had a longer symptom duration than those with less than 286.8 ($p = 0.009$). When we divided the patients according to grade, $mtDNA_{CN}$ and average LTL did not show a statistically significant difference in KOA patients.

Conclusion: Our results that mtDNACN levels may increase to compensate for mitochondrial dysfunction in KOA patients. Average LTL reduction also supported LTL reduction in patients with chronic diseases. These results should be replicated in different and larger ethnic groups.

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P06.022.A Genomic and transcriptomic profiles of osteoporotic vertebral fractures in postmenopausal women

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Background: Osteoporotic vertebral fractures (OVF) represent a major health concern in aging postmenopausal women. This pilot study aimed to characterize the unique genomic and transcriptomic profiles associated with OVF compared to non-osteoporotic controls.

Material and methods: Bone tissue samples were acquired from the vertebral bodies during surgery on postmenopausal women with OVF (n = 41) and those undergoing elective surgery of the thoracolumbar spine without osteoporosis (control, n = 29). DNA was extracted from whole blood and genotyped using the Illumina InfiniumTM Global Screening Array-24 v3.0 BeadChip. Pilot association analysis was performed using PLINK2 with genomic control-corrected p-values. RNA was extracted from vertebral bone tissue and sequenced using Illumina NovaSeq6000 as 150-bp paired-end reads. RNASeq-based differential expression and gene ontology analyses were performed (TMM normalization) comparing OVF and control samples.

Results: The OVF and control groups did not differ in age, BMI, or prevalence of concomitant diabetes, cancer, hyperuricemia, dyslipidemia, hypertension, inflammation, smoking status or medication. Top nominally associated single nucleotide polymorphisms included variants in *VIT, ANK3, SCN9A* and multiple regulatory intergenic regions. Transcriptomic analysis revealed 243 differentially expressed genes after false-discovery rate correction, with 231 downregulated and 13 upregulated in OVF. Gene ontology analysis identified enrichment in extracellular matrix organization, skeletal system development, collagen fibril organization and osteoblast differentiation (all Bonferroni-corrected $p < 10^{-4}$). KEGG pathway analysis showed the top enriched pathways differentiating OVX from controls to be extracellular matrix-receptor interaction ($q = 3.11 \times 10^{-15}$), protein digestion and absorption ($q = 1.47 \times 10^{-10}$).

Conclusion: This study has identified a distinct genomic and transcriptomic signature associated with OVF.

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P06.023.B A new multisystem disorder, TIMES syndrome, reveals unexpected roles of Volume-Regulated anion channels

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We describe two individuals with a congenital disorder including Teleangiectasia, Intellectual disability, Microcephaly, Metaphyseal bone dysplasia, Eye anomalies and Short stature (TIMES syndrome) and a specific facial appearance. The unrelated patients had different never-seen de novo variants (a missense and a fs/stop) in a region of the *LRRC8C* gene, encoding the boundary between the pore and a cytoplasmic domain, that is depleted of sequence variations in control subjects. The LRRC8C gene codes for a subunit of Volume-Responsive Anion Channels (VRACs) channels of the cell membrane that are activated by external hypotonic conditions and allow the cell to discharge water and solubles and protect itself from osmotic swelling. Although molecular structure and functioning of VRACs are well known, their specific physiologic roles in the body have remained elusive. We found that LRRC8C proteins with the patients' variants were incorporated into mature VRACs but the VRACs showed destabilization of subunit interaction and, importantly, enhanced activation, resulting in channel activity even at isotonic conditions in which wild-type channels are closed. The pathogenetic chain between constitutive VRAC activation and the pleiotropic clinical phenotype is still unclear at present; potentially affected cell types are endothelial cells, neurons in the developing brain, growth-plate chondrocytes, and others. Thus, the TIMES syndrome is a pleiotropic, clinically recognizable condition produced by mono-allelic dominant LRRC8C variants leading to constitutional activation and gain-of-function of VRAC channels. It highlights first links between VRAC function and health and disease and might be amenable to pharmacologic treatment in the future.

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P06.024.C Silent doesn't mean harmless: pathogenic role of a synonymous *WRN* variant

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Background: Werner Syndrome (OMIM:277700) is a rare progeroid disorder caused by biallelic pathogenic *WRN* variants, disrupting RecQ helicase function essential for DNA repair. Here, we present a synonymous *WRN* variant in a 25-year-old proband from Turkish Werner Syndrome Registry.

Clinical Report: The proband presented with early-onset diabetes-mellitus, with additional findings of early hair greying, bird-like facies, and hepatosteatosis—features raising suspicion for premature aging. Clinical exome sequencing identified a novel homozygous synonymous *WRN* variant (c.504G>A, p.Lys168=), residing at the last nucleotide of exon 5. Suspecting a splicing defect, we performed cDNA sequencing, yet intriguingly, no aberrant splicing was detected. However, RT-PCR expression analysis revealed a striking 99.6% reduction in mRNA levels, indicating transcript degradation via nonsense-mediated decay (NMD), with only a small fraction escaping NMD and being amplified in cDNA sequencing. To further elucidate the pathomechanism, expression studies following NMD inhibition are currently planned.

Discussion and Conslusion: This study highlights the importance of recognizing clinical signs of premature aging in patients with atypical presentations as a potential early manifestation of premature aging syndrome but also challenges conventional assumptions about synonymous variants as de facto benign. To the best of our knowledge, this is the first *WRN* synonymous variant with proven pathogenicity, highlighting how silent mutations can have consequences. Recognizing these noncanonical disease mechanisms underscores the necessity of a comprehensive approach that integrates clinical, genetic, and functional insights. This enables timely diagnosis, optimizes patient management, and contributes to the prevention of complications in rare aging disorders such as Werner Syndrome.

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P06.025.D Deciphering the phenotypic spectrum associated with *MIA3*-relaed odontochondrodysplasia

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Odontochondrodysplasia (ODCD) is a rare skeletal dysplasia characterized by short stature, skeletal deformities, and dentinogenesis imperfecta (DI). Although the majority of cases were associated with biallelic variants in *TRIP11*, one study described a homozygous truncating variant in *MIA3*, encoding TANGO1, in four sibs with ODCD in association with insulin-dependent diabetes, hearing loss, obesity, and intellectual disability. Subsequently, a homozygous truncating variant in the luminal domain of TANGO1 was identified in a fetus with a lethal skeletal dysplasia and fetal hydrops. Herein, we describe two unrelated patients with a distinct phenotype including severe short limbs, short stature, metaphyseal dysplasia, dysmorphic facies, lax joints, and DI. Other variable features were scoliosis, squint, and cardiac problems. Exome sequencing revealed two homozygous *MIA3* variants in the luminal domain of TANGO1, c.354+2T>G and p.Cys38Phe. The c.354+2T>G variant was confirmed by investigating the patient's mRNA to result in exon 3 skipping and an inframe deletion of 28 amino acids. Our patients lacked the extra-skeletal manifestations noted in the four sibs with *MIA3* variant. However, they had more severe skeletal deformities closely resembling those observed in patients with *TRIP11* variants. Our study suggests the presence of a phenotypic spectrum associated with *MIA3* variants including ODCD with milder skeletal deformities, a classic ODCD with severe skeletal deformities, and a lethal skeletal dysplasia at the severe end of the spectrum. Although the striking phenotypic variability appears to be related to the type and or the location of the *MIA3* variants, the influence of other factors cannot be ruled out.

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P06.026.A Polycystic bone disease: a new family

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Simple bone cysts are benign fluid-filled lytic lesions, which can be unicameral (UBC) or partially separated. UBC can be asymptomatic and typically resolve spontaneously or they can cause pathological fractures. Only one case of familial polycystic bone disease (PCBD), associated with recurrent fractures, has been described to date (PMID: 10457258, 10703941). Here, we report a second familial case, providing new insights into this ultra-rare disease. Patient 1 presented at 16 years of age with recurrent fractures (left femur and, subsequently, left tibia). Skeletal radiological survey, CT and MRI confirmed the presence of multiple bone cysts (femurs, tibias, calcaneus and ulna). Pamidronate treatment showed no efficacy and she had multiple orthopedic interventions (intra-medullary nails, bone graft). Histological examination revealed a thin, fibrous cyst wall with no epithelial lining, consisting of fibroblasts and irregular bands of fibrin-like material mimicking odontogenic cement. She did not show any other health problem (normal stature, vision and hearing) except steatotic liver disease and polycystic ovary syndrome. Her father had a fracture of his upper limb and multiple cysts of long bones. Her younger brother showed a similar radiological picture. Genetic assessment including gene panel for skeletal dysplasias (including *FGFR1*), chromosomal microarray and quatuor whole genome sequencing, could not characterize the cause of this condition. In conclusion, we

report the second case of PCBD, providing new insights into the clinical, radiological and pathological characterization of this ultra-rare disease. We hope that the report of further cases will let the characterization of the molecular basis of this condition.

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P06.027.B Syndrome-informed phenotyping reveals a polygenic basis for connective tissue disorder variation in the general population and identifies novel loci for aortopathies

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Background: Thoracic aortic aneurysms and dissections (TAADs) are a class of life-threatening aortic pathologies that can be syndromic (e.g., Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and vascular Ehlers-Danlos syndrome (vEDS)) or non-syndromic. Compared to syndromic TAADs, non-syndromic TAADs are more common but less understood and thus harder to screen.

Material and methods: Following previous work that has suggested Mendelian disorders are phenotypic extensions of the general population, we use facial shape to establish axes of variation between controls (N = 8,246) and individuals with MFS, LDS, and vEDS. We then subject control scores along these axes, or syndrome-informed phenotypes (SIPs), to genome-wide association studies. Next, we investigate whether the discovery of TAAD (i.e., ascending aorta diameter) loci can be boosted by leveraging pleiotropy with SIPs via the conditional false discovery rate (FDR). We additionally implement the conjunctional FDR to examine shared loci between TAADs and SIPs.

Results: We recovered 32, 50, and 11 genome-wide significant loci for the LDS, MFS, and vEDS SIPs, respectively, which were primarily enriched for processes related to embryonic and skeletal system development, as well as limb morphogenesis. The loci common to all SIPs implicate key craniofacial transcription factors, including known loci near *TBX15*, *KLF4*, and the *HOXD* cluster, as well as *DCHS2*, which likely also regulates cardiomyocyte proliferation and cardiac growth. Using the FDR (1%) approaches, we found 112 novel loci for TAAD, 46 of which were shared across all SIPs.

Conclusion: Our results show that the face can yield novel genetic insights into TAADs.

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P06.028.C Severe malnutrition masking a well-defined skeletal dysplasia: a case of achondroplasia with coexisting *BRAF* variant

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Background: Rare diseases, which currently exceed 7,000 in number and constitute a significant workload in Medical Genetics clinics, affect approximately 6% to 8% of the worldwide. Although distinct dysmorphic features can guide specialists toward a specific diagnosis, presence of overlapping findings across different diseases, blended phenotypes, and environmental factors etc. pose challenges in establishing a clinical diagnosis.

Methods-Results: A female proband, followed for prenatal long bone shortening, was born to non-consanguineous parents with an unremerkable family history. Postnatally, she exhibited severe growth and developmental delay. At 7-months-old, physical examination revealed that height: 52.5 cm (SDS: −5.73), weight: 3800 gr (SDS: −5.61), head circumference: 41.5 cm (SDS: −1.34), hypotonia, frontal bossing, flattened nasal bridge, macrostomia, severe loss of adipose tissue and rhizomelia. Radiographs showed iliac bone hypoplasia, epi-metaphyseal changes in the femur, and loss of vertebral body height in the lumbar spine. In a case that lipodystrophy and skeletal dysplasia were prominent, c.1138G>A(p.Gly380Arg) in FGFR3 consistent with achondroplasia and c.1774A>G(p.Ile592Val) in BRAF heterozygous changes were detected in the clinical exome sequencing. Segregation study is ongoing.

Conclusion: Here, we present how severe malnutrition can obscure a well-defined syndrome such as achondroplasia and complicate clinical diagnosis. BRAF V600E mutation is known to influence plasma lipid profiles (PMID: 35565240). BRAF mutation identified in our case has been previously associated with cancer; however, its relation with lipid metabolism remains unclear. This case underscores the importance of comprehensive genomic analysis in atypical presentations and highlights the potential interplay between genetic and environmental factors in shaping phenotypic outcomes.

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P06.029.D Long-read sequencing cracks unsolved cases and further improves genome diagnostics in epidermolysis bullosa

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Background: Standard genetic diagnostics of Epidermolysis Bullosa (EB) using short-read sequencing has a diagnostic yield of ~95%, leaving 5% of patients in uncertainty about their genetic diagnosis. Here, we applied Oxford Nanopore Technologies long-read sequencing (LRS) to diagnose unsolved cases.

Material and methods: Nine patients were selected with either no causal variant identified or only one variant in a presumed recessive form of EB. Libraries were generated and 24 EB-related genes were sequenced.

Results: In four patients, intermediate-sized (2.5–8 kb) multi-exon deletions were detected that were not found in public databases. Two *COL7A1* deletions were found in patients with dominant dystrophic EB (DDEB) in whom no previous variant was

identified, explaining the phenotypes. The same *ITGB4* deletion was identified in two patients. For one patient with junctional EB-localized, a heterozygous pathogenic frameshift variant had been identified previously, completing the genetic diagnosis. In the other patient, no additional variant was found, leaving the EB simplex-localized phenotype yet unexplained. In a fifth patient with DDEB, we found a likely pathogenic intronic splice-variant in *COL7A1*. RT-PCR confirmed alternative splicing leading to the in-frame insertion of seven amino acids to the triple-helical region. The variant was found to co-segregate with the disease in the patient's family.

Conclusion: Long-read sequencing yielded a genetic diagnosis for four of nine patients, highlighting its added value to EB diagnostics. Interestingly, four of five variants identified were intermediate-sized deletions, a class of variants that is missed with routine diagnostics due to shortcomings of current whole exome sequencing methods.

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P06.030.A Study of the splicing variants in the *FBN1* gene of patient with Marfan syndrome

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Research Centre for Medical Genetics, Moscow, Russian Federation.

Background: Marfan syndrome is caused by pathogenic variants in the *FBN1* gene, primarily through two main mechanisms: haploinsufficiency, caused by loss-of-function variants associated with an increased risk of aortic complications, and dominant-negative effects, resulting from missense variants or in-frame deletions/insertions. Splicing variants can contribute to disease pathology through either mechanism, and elucidating the underlying mechanism requires experimental investigation of the specific splicing defects they induce.

Material and methods: Variants in *FBN1* were identified in patients with Marfan syndrome through either targeted NGS panel or WGS sequencing. Splice-altering variants were predicted using SpliceAI. Total RNA was isolated from patient-derived fibroblasts using the Trizol method. RT-PCR followed by deep next-generation sequencing was performed to detect splicing alterations and allelic imbalance.

Results: We studied a cohort of 230 Marfan syndrome patients diagnosed by clinical criteria, carrying pathogenic or VUS variants in *FBN1*. SpliceAI predicted the potential impact on splicing for 42 variants. We performed splicing analysis on patient-derived RNA samples isolated from fibroblasts for 15 variants: 11 splice-site variants, 2 exonic variants, and 2 deep intronic variants. Numerous splicing alterations were analyzed in the context of their impact on reading frame preservation or disruption. The resulting interpretation of splicing variants was correlated with the clinical presentation of the patients.

Conclusion: Splicing variants in *FBN1* require further study to clarify their pathogenic mechanisms. Improved understanding will enhance genotype-phenotype correlations and advance diagnostic and therapeutic approaches for Marfan syndrome patients.

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P06.031.B Exploring the genetics of molar incisor hypomineralization (MIH): a molecular study on candidate genes involved in human dental enamel development

Simone Frisari[1], *Stefania Zampieri*[2], *Valentina Luppieri*[2], *Maria Pina Concas*[2], *Beatrice Spedicati*[1,2], *Aurora Santin*[1,2], *Alessandro Pecori*[2], *Milena Cadenaro*[1,2], *Giorgia Girotto*[1,2]

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Background: MIH is a worldwide-spread qualitative developmental defect of dental enamel. It affects some or all permanent molars and incisors, with variable colour demarcated opacities. From a genetic perspective, MIH is a multifactorial disorder, and, to date, its precise aetiology remains unclear and contentious.

Methods: Eighteen subjects aged 6 to 18 years attending the dental clinic of the IRCCS Burlo Garofolo (Trieste, Italy) and affected by MIH, diagnosed according to Lygidakis et al. (PMID:34669177), were enrolled in the study. Genetic analysis was performed by Whole Exome Sequencing (WES).

Results: Upon WES analysis, in 9/18 patients, 7 novel heterozygous missense variants were identified in the following genes: *HMCN1*, involved in dentinogenesis; *MMP2* and *TFIP11*, implicated in enamel formation; *LAMB3*, associated with amelogenesis imperfecta; *TTLL12* and *RMI1*, linked to MIH. Moreover, in two affected siblings, a large pathogenic deletion was identified in the *ENAM* gene, encompassing the first four exons. ENAM plays a crucial role in enamel development and pathogenic variants within *ENAM* gene were reported to cause enamel hypomineralization.

Conclusions: For the first time in Italy, we deepened the genetic bases of MIH, identifying novel variants in genes involved in enamel formation and development. This work emphasises the significance of genetic investigations for disease characterisation and prevention, and the choice of a suitable treatment for each patient, thereby enhancing their quality of life. In the future, it may be possible to identify new genes implicated in the condition by expanding the patient cohort and involving more Italian centres in the study.

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P06.032.C Evaluation of clinical and molecular features and oxidative stress parameters in ciliopathies with skeletal Involvement

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Background: Ciliopathies encompass a broad spectrum caused by variants in genes associated with ciliogenesis and ciliary trafficking. Among these, ciliopathies with predominant skeletal involvement are classified as a subgroup of genetic skeletal disorders. The aim of our study was to clarify the molecular etiology in patients with skeletal ciliopathies, establish genotype-phenotype

correlation, and investigate the relationship between ciliary dysfunction and oxidative stress.

Material and methods: A total of 20 patients with a preliminary diagnosis of skeletal ciliopathy were included in the study and whole exome sequencing was performed to elucidate the molecular etiology. Additionally, oxidative stress parameters were compared between the patient and control groups.

Results: Molecular etiology was identified in 16 (80%) of the patients, while no causal variants were detected in four (20%). Based on clinical and molecular findings, six patients were diagnosed with Ellis-van Creveld syndrome, six with oral-facial-digital syndrome, five with short-rib thoracic dysplasia, one with Meckel-Gruber syndrome, one with cranioectodermal dysplasia, and one with an undefined ciliopathy with skeletal involvement. The most frequently detected variants were in the *EVC* gene, followed by *DYNC2H1*. Among the identified variants, nine novel variants were described in the *EVC*, *EVC2*, *DYNC2H1*, *FAM149B1*, *KIAA0586*, *IFT80*, and *WDR35* genes. Oxidative stress parameters were found to be elevated in the skeletal ciliopathy patient group compared to the control group; however, statistical analyses did not show a significant difference.

Conclusion: This study has detailed the clinical, radiographic, and molecular characteristics of skeletal ciliopathies and contributed to the expansion of the mutational spectrum.

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P06.033.D A longitudinal study to evaluate the advantages of HR-pQCT to assess bone mineral density in patients with Osteogenesis Imperfecta

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IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy.

Background: Osteogenesis Imperfecta (OI) is a rare disease characterized by bone fragility. This ongoing prospective study aims to test if High-Resolution peripheral Quantitative Computed Tomography (HR-pQCT), a high-resolution low-dose technique, can complement the current standard for quantitative bone assessment, i.e. areal bone mineral density(aBMD) measured by Dual-energy-X-ray Absorptiometry(DXA).

Methods: DXA scans are performed at lumbar spine, hip, total body, and forearm. HR-pQCT images are acquired at distal radius and tibia, measuring volumetric BMD(vBMD) and microarchitectural parameters.

Results: To date, 35 patients have been enrolled: 6 children, 29 adults; 19 females, 16 males; 32 OI Type I, 2 Type III and 1 Type IV. For 21 patients 12-months follow-up has been performed, data analysis is ongoing. Preliminary results demonstrate significant site-specific correlations of DXA-aBMD with HR-pQCT-vBMD ($r = 0.89$) and HR-pQCT cortical parameters (cortical vBMD, $r = 0.83$, cortical thickness $r = 0.87$) at the ultradistal radius. Cortical areas measured by HR-pQCT, at both radius and tibia, are correlated with DXA-aBMD at all sites. However, there is almost no significant correlation between DXA-aBMD (all sites) and trabecular HR-pQCT parameters (vBMD, number, thickness, separation).

Conclusion: There is preliminary evidence that DXA-aBMD cannot explain HR-pQCT trabecular measurements and may thus not be able to identify specific trabecular bone phenotypes/deficits in OI patients. HR-pQCT imaging therefore warrants further testing to identify patients at higher risk of fracture and target therapies. With this aim, ERN BOND supported the establishment of an international working group for the development of HR-pQCT standardized protocols and guidelines for the clinical management of OI patients.

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P06.034.A Whole-body MRI in a cohort of adult patients affected by Nurofibromatosis type 1: suggestions for appropriate follow-up

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Background: The care of adults with genetic disorders has been under the radar due to improved survival rates, but specific guidelines are often lacking. Neurofibromatosis type 1 (NF1) patients require life-long care for cancer screening, and current guidelines recommend a whole-body MRI (wbMRI) during transition from adolescence to adulthood. However, this is still not standard-of-care in many centers and there are no clear indications regarding how often to repeat it. We hereby describe our recent experience in Pavia, where collaboration with the radiologists allowed to perform wbMRI for the first time in a cohort of adult NF1 patients.

Material and methods: Since July 2024, we recruited adult NF1 patients routinely followed at the Medical Genetics Department of San Matteo hospital in Pavia for wbMRI. All imaging results were reviewed by a multidisciplinary team composed of clinical geneticists and radiologists to establish appropriate follow-up.

Results: 16 patients were recruited (3F/13M, mean age 37 years) and a wbMRI was performed for 11/16 patients.Plexiform neurofibromas (PNs) were identified in 7/11 patients (63%) and in most cases (6/7; 86%) new previously unknown internal PNs were detected. Based on multidisciplinary evaluation, a follow-up wbMRI was suggested for four patients (4/7; 57%) while either ultrasound or targeted MRI were planned for the others. In one patient wbMRI identified an unexpected lung adenocarcinoma allowing for timely surgery.

Conclusion: WbMRI is a valuable tool in assessing internal tumor burden, a precious information considering the newly available targeted therapeutic strategies (i.e. MEK-inhibitors) in NF1.

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P06.035.B The dual role of IRF7 in immunodeficiencies and autoinflammatory diseases

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Background: The role of Interferon Regulatory Factor 7 (*IRF7*) is well established in recessive immunodeficiencies due to loss of function mutations. However, gain of function mutations in this gene are not described despite the fact that the pro-inflammatory function of this gene is well known.

Material and methods: Exome analysis in 198 autoinflammatory patients, negative for variants in known genes and 8,037 patients coming for different clinical questions.

Results: In one autoinflammatory family (0,5%) we identified a *IRF7* truncating variant c.738_750delCGGGCCCCAGCCC, p.Gly247fs, segregating in the proband with Still disease and in affected members. Transactivation assay demonstrates that removing of C-terminal domain from position 246 results in 100-fold increase activity. We identified 2 additional close *IFR7* truncating variants in 6 patients from a larger cohort, coming for different clinical purposes: c.749_750delCC, p.Pro250fs and c.839delC, p.Ala280fs. Active recontact of those patients showed the presence of joint pain, diarrhoea or arthritis (often requiring knee prosthesis) in all of them.

Conclusion: We report here new *IRF7* variants in autoinflammatory patients, which suggest to include *IRF7* in either autosomal dominant autoinflammatory or autosomal recessive immunodeficiency diseases depending on loss/gain variant effect. Since *IRF7* is unable to stimulate interferon gene expression in vitro in the absence of virus infection, we believe that the variability of clinical outcome in patients is strongly dependent on environmental factors such as the timing and type of viral infections. Further validation in larger cohort of patients is necessary to confirm the exact prevalence of *IRF7* variants in autoinflammatory disorders.

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P06.036.C Diagnostic challenges in skeletal dysplasia: a clinical overview based on a 10-year retrospective study of single genetic department

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Background: Genetic skeletal dysplasia is heterogeneous group of 771 disorders associated with 552 genes affecting bone and cartilage. Given that some skeletal dysplasia can be caused by several different genes, and also that some genes can cause a variety of different phenotypes, achieving a molecular diagnosis can be challenging.

Materials and methods: Our 10-year retrospective study (2014–2024) includes 252 probands from the skeletal dysplasia database of Department of Clinical Genetics, University Children's Hospital from Belgrade, Serbia. We conducted a clinical overview of tested probands with analysis of the diagnostic approach.

Results: Out of 252 probands, 217 were tested using next-generation sequencing (clinical exome sequencing or whole exome sequencing in first step, and whole genome sequencing in second step in selected negative probands) and 35 were tested using targeted gene/mutation analysis. Next generation sequencing showed a high detection rate of the causative gene variant(s) of 61.21% (131/217). Additionally, in 5.52% (12/217) of a non-diagnosed probands gene variant(s) of unknown significance were identified. In the targeted tested group, result was positive for 75.67% (27/35) of them. We monitored whether the diagnosis was made with the first genetic test or if more steps were required. Some of the probands went through diagnostic odyssey before establishing the molecular diagnosis.

Conclusion: Our results showed that combination of next generation sequencing and gene/mutation targeted testing could achieve a high diagnostic rate for probands with skeletal dysplasia. We learned from analyzing diagnostic challenges in patients in whom the diagnosis was achieved with difficulty.

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P06.037.D Unraveling the genetic landscape of rare Ichthyosis in Tunisian patients: advancing molecular diagnosis and uncovering novel variants through whole exome sequencing

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Background: Over 50 genes are associated with ichthyoses, contributing to the broad spectrum of clinical manifestations. The genetic diversity and overlapping phenotypes raise challenges in diagnosing ichthyoses in many Tunisian patients. This study aimed to assess the effectiveness of Whole Exome Sequencing (WES) in identifying genetic variations in Tunisian patients with different subtypes of ichthyosis to improve molecular diagnosis and management.

Material and methods: WES was performed on 46 patients suspected of having various forms of ichthyoses.

Results: Mutations were detected in 38 (83%) patients, while no variants were found in 8 (17%) patients. Overall, variants were identified in 14 distinct genes. In a subgroup of 23 patients with autosomal recessive congenital ichthyosis (ARCI), 4 variants were identified in *CYP4F22*, 2 in *NIPAL4*, 1 in *CERS3*, 1 in *ALOXE3*, 1 in *ALOX12B*, and 5 in *TGM1* gene. A new variant in the *KRT1* gene was discovered in a patient with epidermolytic hyperkeratosis. In contrast, another patient with the same phenotype had a heterozygous pathogenic c.467G>A (p.Arg156His) variant in the *KRT10* gene. Twelve patients carry *LORICRIN*, *ABHD5*, *SLC27A4*, *RAG1*, *FLG* and *STS* genes variants, associated with syndromic and

commun ichthyosis, whereas they were clinically suspected of having the ARCI phenotype.

Conclusion: The high detection rate of pathogenic variants (83%) highlights the utility of WES in improving molecular diagnosis. Additionally, discovering new variants highlights the importance of population-specific genetic characterization, which deepens the understanding of the genetic landscape of ichthyoses, aiding in better diagnostic accuracy and the potential development of targeted therapies.

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P06.038.A Systematic use of WES improves the diagnostic yield for skeletal dysplasia conditions within the national healthcare system

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Background: Skeletal dysplasia (SD) is a large and heterogeneous group of rare genetic disorders that affects bone and cartilage growth. These disorders are diagnosed on the basis of clinical, radiographic, and molecular criteria. We present the yield of genetic testing and molecular epidemiology of SD in the Slovenian national health care system.

Material and methods: We retrospectively reviewed the records of 470 patients with clinical features of SD, including prenatal, childhood, and adult patients referred to the national genetic reference center over a ten-year period. In 262 patients, next-generation sequencing (NGS) was performed either as whole-exome sequencing (WES) or whole-genome sequencing (WGS). Targeted sequencing was performed in 208 patients with a clear-cut clinical diagnosis.

Results: A definitive genetic diagnosis using NGS was achieved in 50% (n = 131) of patients. Among the positive cases, 49.61% initially presented with a nonspecific diagnosis of SD and genetic testing contributed to establishing the diagnosis. Moreover, we demonstrated high genetic heterogeneity in our SD cohort, with 66 distinct causative genes, resulting in different types of SD. We detected 132 SD-causing variants, of which 29 were novel.

Conclusion: With the systematic use of WES and WGS, we significantly improved the diagnostic yield of SD in the Slovene national health care system. Moreover, we found significant genetic heterogeneity and reported the genetic epidemiology of SD in the Slovenian population.

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P06.039.B Mitochondrial skeletal disorders: a retrospective cohort of 9 French cases

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Background: Mitochondrial disorders (MD) result from primary mitochondrial dysfunction leading to multisystemic manifestations. However, bone disorders are uncommon in MD. A group of autosomal recessive skeletal dysplasias linked to mitochondrial dysfunction is yet emerging, involving *LONP1*, *HSPA9*, *IARS2*, *PAM16*, *AIFM1* and *PISD* genes. We aim to describe and compare the skeletal presentation of these mitochondrial skeletal disorders (MSD).

Material and methods: We recruited 9 cases (6 families), including 5 females and 4 males of diverse ethnicities; 4 fetuses and 5 patients (8 to 15 years old).

Results: We identified biallelic *LONP1* mutations in 3 fetuses and 1 patient, *AIFM1* (2 brothers) and *PISD* (1 fetus; 2 patients). Common symptoms include growth retardation and absence of microcephaly. *PISD* fetus displayed neuronal alterations, platyspondyly, short ribs, curved femora and metaphyseal spurs. *LONP1* fetuses, but not the living patient, harbour cardiopathies and diaphragmatic hernias. All MSD patients display motor developmental delay, eye abnormalities, spondyloepimetaphyseal dysplasia and maxillofacial anomalies. Distal femoral epiphysiodesis and patellar dislocation affect the *LONP1* patient. *AIFM1* and *PISD* patients display brachy-metacarpy/-phalangy. Metaphyseal alterations and enlarged knees are observed in *AIFM1*, whereas joint dislocations and coxa vara are seen in *PISD*.

Conclusion: Growth retardation, severe epiphyseal dysplasia, spine involvement and maxillofacial anomalies are common to MSD. Together with the shared extra-skeletal signs, this supports a common clinicoradiological presentation linked to genes involved in mitochondrial protein homeostasis and transport. However each genotype displays some specific signs: we recommend to assess joint laxity in *LONP1* and coxa vara in *PISD*.

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P06.040.C Not only reverse phenotyping: reverse genotyping in Bazex-Dupré-Christol syndrome

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Background: The ACMG standards and guidelines for reporting copy number variations provide for not considering those that are small, inherited, and not containing OMIM disease-genes. Although generally valid, these criteria have limitations such as in cases due to small duplications falling in desert-gene/intergenic regions, which regularly escape filtering for clinical reporting. Bazex-Duprè-Christol syndrome (BDCS) is an X-linked dominant genodermatosis recently associated with a ~100 Kb microduplication spanning between the IGSF1 and ARHGAP36 genes in Xq26.1. Before the identification of its genetic cause, BDCS has been defined by the combination of congenital hypotrichosis, atrophoderma follicularis and basal cell carcinomas.

Material and methods: Two unrelated families with multiple affected individuals presenting atrophoderma follicularis of dorsum of hands/elbows/knees, facial milia and hypotrichosis and acrocordons underwent genetic testing for an unclassified genodermatosis. In both families the phenotype was transmitted from the affected mother.

Results: After initial negative results at SNParray, precise clinical diagnosis guided re-analysis and prompted to the identification of the recurrent microduplication in Xq26.1 in both families. Affected individuals did not present basal cell carcinomas, while facial milia and atrophoderma follicularis were the only features occurring with full penetrance.

Conclusion: Presented families illustrate the crucial role of deep phenotyping before prescribing molecular testing in apparently attenuated presentations such as BDCS. Our experience put forward the opportunity to identify in atrophoderma follicularis, facial milia and lack of male-to-male transmission the minimum set of criteria for array-based data re-analysis in the suspicion of BDCS.

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S. Morlino: None. **M. Capurso:** None. **O. Palumbo:** None. **M. Castori:** None.

P06.041.D Unraveling novel genetic markers and transcriptional regulator of osteoporosis

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Background: Osteoporosis is the most common bone-related disease, driven by a combination of environmental and genetic factors. The aim of our study is to investigate the underlying gene expression mechanisms and characterize the genetic profile of affected individuals in order to identify correlations between genetic background and osteoporosis symptoms, low bone mass and osteoporotic fractures.

Material and methods: We analyzed gene expression profiles in bone samples from 75 osteoporotic and 25 control post-menopausal Greek women. Bone mineral density (BMD) was measured using dual X-ray absorptiometry (DXA), and serum bone markers were assessed. Total RNA was isolated from bone tissue and analyzed via TGFβ-BMP signaling pathway microarray. Additionally, we performed ChIP-seq binding analysis at loci associated with low bone density and increased fracture risk.

Results: The gene expression analysis revealed significant molecular distinctions between osteoporotic patients and controls. Specific SNPs, including rs3736228 (LRP5) and rs1286083 (RPS6KA5), were linked to reduced BMD, whereas rs6465508 (SHFM1) and rs7851693 (FUBP3) correlated with higher fracture risk. Notably, the CTCF-dependent SNP in *DKK1* emphasize genetic influences on bone health and remodeling. Lastly, overexpression of *CDKN1A* in peripheral blood and bone tissue underscores its role in osteoporosis pathophysiology. Elevated *ENG* expression at fracture sites suggests its involvement in healing processes, while increased *FOS* levels in peripheral blood highlight its potential as an early diagnostic biomarker.

Conclusion: Genetic profiling in osteoporosis can uncover key biomarkers for early diagnosis. Knowledge of specific SNPs that affect gene regulation can pave the way for personalized approaches in treating bone diseases.

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P06.042.A Homozygous LRP6 variant in a patient with multiple birth defects and ectodermal dysplasia

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Background: *LRP6* plays a crucial role in embryogenesis, cell signaling, and tissue development processes. Heterozygous loss-of-function variants in *LRP6* gene have been associated with non-syndromic dental agenesis, while some heterozygous and homozygous missense mutations have been linked to increased risk of coronary artery disease. Notably, homozygous loss-of-function variants have never been reported in human population. However, experimental studies on mouse embryos have demonstrated that homozygous mutations can result in a complex phenotype encompassing multiples congenital anomalies and early mortality.

Material and methods: we report a case of an 8-year-old male with a complex phenotype characterized by ectodermal dysplasia, bilateral cleft lip and palate, split hand-foot and vertebral anomalies. A panel of genes associated with ectodermal dysplasia and hypodontia was analyzed by next generation sequencing.

Results: a homozygous variant in *LRP6* gene was identified (NM_002336.3: c.2280-3T>G). The variant has never been reported in the literature nor in the available databases, is not present in databases aggregating exome and genome sequencing data from large-scale sequencing projects, and is predicted to alter the transcript splicing by multiple lines of computational evidence.

Conclusion: the clinical characteristics of our patient overlap the phenotypic spectrum observed on *lrp6* knock-out animal models; however, until now, no other human patients carrying homozygous splicing variants in this gene have been described in the literature. Ongoing functional studies aim to characterize the effect on splicing and confirm the pathogenicity of the variant detected in our proband.

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P06.043.B Is mild bone fragility genetic? - Genetic findings in a cohort of patients with early-onset fractures and/or osteoporosis and no extra-skeletal signs of osteogenesis imperfecta or significant bone deformities

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Background: Except for monogenic disorders with significant fractures and/or extra-skeletal features, such as osteogenesis imperfecta (OI), bone fragility is usually considered a multifactorial disorder. Our aim was to molecularly evaluate a cohort of patients with mild bone fragility in order to identify its monogenic causes and/or genetic risk factors.

Material and methods: We evaluated a cohort of 38 unrelated patients with bone fragility and absence of extra-skeletal signs of OI or significant bone deformities. Genetic analysis was performed using a skeletal dysplasia NGS panel and MLPA for *COL1A1* and *COLA1A2*.

Results: The cohort included 22 females and 16 males. Twenty patients were children or adolescents and 18 were adults. A monogenic cause was identified in three patients (7.9%), namely a duplication of exons 6 to 9 of *PLS3*, a complete deletion in mosaic state of *COL1A1*, and a frameshift variant in *LRP5*. No relevant variants were detected in 24 cases (63.2%). In the remaining cases (9/38, corresponding to 23.7%), variants that have been previously associated with increased risk of recurrent fractures and/or osteoporosis (including common polymorphisms) were found, namely *LRP5*, *WNT1*, and *DKK1*. Six patients carried more than one such variants.

Conclusion: These results highlight that mild bone fragility can, in rare cases, be monogenic, which is important for genetic counselling. While knowledge of genetic risk factors contributing to bone fragility is increasing, they explain only a portion of cases, indicating that other genetic and environmental factors should be involved.

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P06.044.C Probable founder effect for rare variants of the *P3H1* gene in Bashkirs (Russia)

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Background: Prolyl-3-hydroxylase-1 (P3H1) is part of a complex that hydroxylates proline residues in type 1 collagen. About 70 pathogenic variants in the *P3H1* gene are linked to autosomal recessive forms of osteogenesis imperfecta (OI) type III/VIII. This paper reports three *P3H1* gene variants identified in Bashkir patients with OI and evaluates their prevalence in Bashkir ethnic groups.

Material and methods: The study included 65 OI patients, 125 family members, and 731 individuals of Bashkir ethnicity from six districts in Bashkortostan, along with Bashkirs from other regions of Russia. NGS sequencing of a target 664-gene panel was performed on the DNBSEQ-G400 platform, validated by Sanger sequencing. Three *P3H1* gene variants were screened using TaqMan technology.

Results: Two unrelated Bashkir families with OI were identified: one child with a homozygous c.1051G>T (p.Glu351*) mutation in *P3H1* died at age of 3 months, while another patient had a non-lethal form with compound-heterozygous variants c.1051G>T (p.Glu351Ter) and 1948G>A (p.Gly650Arg). A heterozygous c.1720+4G>A variant was found in a patient not fitting OI type VIII. Screening in the Bashkir population three *P3H1* detected variants revealed the 1948G>A variant in one individual in a heterozygous state, while c.1051G>T (p.Glu351Ter) appeared in three unrelated individuals, indicating a potential founder effect. The 1720+4G>A variant had a frequency of 0.007, significantly higher than in available databases.

Conclusion: These results underscore the importance of studying the genetic basis of OI across ethnic groups to identify specific pathogenic variants. Further research on their prevalence will clarify their clinical significance in OI formation.

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P06.045.D Investigating the pre- and postnatal phenotypic spectrum and prognosis of ARCN1-related disorder

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Background: *ARCN1*-related condition is rare and caused by defective collagen transport. To date, 21 cases were described with a clinical spectrum ranging from fetal death to a milder phenotype including intrauterine growth restriction (IUGR), short stature and micrognathia. The challenge remains to predict the severity and postnatal outcome based on the prenatal phenotype and genotype. We describe a one-year-old girl diagnosed prenatally with a favorable outcome and compare the clinical findings to previously reported cases.

Material and methods: Prenatal Trio-Exome sequencing, full clinical examination and phenotype-genotype comparison based on the literature were performed.

Results: Ultrasound at 24 weeks of gestation showed mild IUGR and rhizomelic shortening. Trio-Exome sequencing revealed a novel de novo *ARCN1*-variant c.1091G>A (p.Trp364Ter), classified as pathogenic (ACMG criteria). The patient was born prematurely with micrognathia and a ventricular septal defect. At one year she had normal development. Including our patient to the previously reported postnatal cohort rhizomelia was observed in 56% (9/16), prematurity in 75% (12/16), development delays in 69% (11/16) and congenital heart disease in 38% (6/16). Additional 6 prenatal cases had been published, which all showed rhizomelia (6/6) and skeletal anomalies in the early second trimester. Outcomes included 3 fetal demises and 3 terminations of pregnancy.

Conclusion: Prenatal lethal or severe cases had a higher proportion of rhizomelia, skeletal anomalies and other ARCN1-related features which were detected earlier during pregnancy than in liveborn cases, including ours. Onset and severity during pregnancy may help to predict prognosis, but further patients are needed to assess genotype-phenotype correlation.

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P06.046.A A novel deep-intronic variant in HES7 unveils a poison exon mechanism underlying spondylocostal dysostosis type 4

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Background: Spondylocostal dysostosis (SCD) is an ultra-rare autosomal recessive disorder caused by pathogenic variants in *HES7*. To date, only six causative variants have been identified, all affecting coding or canonical splicing regions.

Materials and methods: Whole-genome sequencing (WGS) was performed on a patient with clinical features of SCD. The impact of the identified variant was assessed using bioinformatic tools, transcriptomic analysis of publicly available RNA-seq data, and splicing assays in patient and control samples.

Results: WGS revealed a novel homozygous deep-intronic variant in *HES7*, absent from population databases. It is predicted to enhance the strength of a cryptic intronic acceptor site, which would result in aberrant inclusion of a 49 base-pair pseudoexon and the introduction of a premature termination codon. This transcript is likely to be exposed to nonsense-mediated decay (NMD) and to subsequently cause loss of *HES7* function. Transcriptomic analysis revealed that pseudoexon inclusion occurs at low levels in a tissue-specific manner in healthy individuals, suggesting an underlying physiological role as a poison exon. However, splicing assays in lymphocyte-RNA revealed significantly increased pseudoexon inclusion in the patient compared to controls.

Conclusion: This is the first report to implicate a poison exon in the regulation of a somitogenesis-related gene. The patient's homozygous variant likely disrupts normal *HES7* splicing across multiple tissues, leading to biallelic loss of function and the SCD phenotype. These findings expand the genetic spectrum of SCD and emphasize the value of WGS and transcriptomic approaches in uncovering causal non-coding variants.

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P06.047.B Genetic aspects of pediatric genodermatosis cases from a center for rare diseases

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Background: Genodermatoses encompass a broad spectrum of genetic disorders that involve the skin, and may or may not involve other organ systems. Therefore, the clinical identification of uncommon genodermatoses is challenging and many patients remain without a molecular diagnosis, even though more than 500 causative genes have been discovered. The presented cases highlight the importance of interdisciplinary approaches in the diagnosis of rare skin disorders.

Material and methods: More than 20 cases with different dermatological conditions from an interdisciplinary dermatological and genetic consultation for pediatric and juvenile dermatology were examined for the presence of causative molecular genetic changes via (targeted) NGS analysis.

Results: We present a heterogeneous collective of rare pediatric/juvenile genodermatoses with regard to symptomatology and underlying molecular genetic alterations. Among others, we report on a previously undescribed de novo missense alteration in the *NSDHL* gene in an 8-year-old female patient who presented with inflammatory linear verrucous epidermal naevus (ILVEN). Furthermore, we describe a previously undescribed de novo frameshift alteration in the *ATP2A2* gene (Darier's disease) in a 9-year-old female patient who presented with acrokeratosis verruciformis and moderate neurodevelopmental disability.

Conclusion: The described cases show that molecular genetic diagnostics is a useful addition to dermatological or pediatric consultation, which often contributes to timely diagnosis of pediatric/juvenile genodermatoses. This in turn enables the best possible patient care at an early age and can considerably improve treatment outcome.

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P06.048.C Development of an S2k Guideline for Ehlers-Danlos Syndrome (EDS) in Germany, Austria, and Switzerland

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Background: Ehlers-Danlos syndrome (EDS) comprises a group of genetically determined connective tissue disorders characterized by joint hypermobility, skin fragility, and systemic manifestations. Despite the significant burden on affected individuals and the diversity of subtypes, no comprehensive evidence-based guidelines exist in the German-speaking region. To address this gap, the development of an S2k guideline for EDS diagnosis and treatment was initiated under the auspices of the Association of Scientific Medical Societies in Germany (AWMF).

Material and methods: The guideline is being developed under the coordination of the Society for Human Genetics (GfH) in collaboration with 16 professional societies and 4 patient organizations from Germany, Austria, and Switzerland. The process involves reviewing available evidence, conducting expert discussions, structured consensus rounds (e.g., Delphi method), and incorporating feedback from patient organizations. Additionally, recommendations for therapeutic interventions are being drafted in cooperation with physiotherapy experts.

Results: The ongoing guideline development focuses on establishing standardized criteria for EDS diagnosis, including clinical and genetic diagnostics, and creating evidence-based recommendations for medical and therapeutic care. The inclusion of patient perspectives ensures a practical and patient-centered approach. This initiative aims to create a unified standard for EDS care across the German-speaking region.

Conclusion: The development of the S2k guideline represents an essential step toward improving EDS diagnosis and treatment. Its interdisciplinary and patient-focused approach ensures that the final guideline will meet high-quality standards, enhance collaboration among healthcare professionals, and address the needs of affected individuals.

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P06.049.D A novel PIK3R1 germline splice variant in a multi-generation family with overgrowth, vascular malformations, lipomas, and developmental delay

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Background: PIK3R1 encodes a regulatory component of the PI3K/AKT signalling pathway which is critical for growth. Pathogenic germline PIK3R1 variants are associated with SHORT syndrome, characterized by short stature, lipodystrophy, and insulin resistance, or an immune phenotype (Activated PI3K-Delta syndrome) depending on the molecular mechanism. Recently, mosaic PI3KR1 variants have been associated with overgrowth and vascular malformations. We present a 3-generation family with a variable phenotype associated with neurodevelopmental, cutaneous and growth issues with a germline variant previously reported in PIK3R1-related mosaic overgrowth.

Material and methods: Trio exome sequencing of a family with vascular malformations, lipomas, macrocephaly, and neurodevelopmental issues was performed. Gene-specific RNA-sequencing of the proband was also performed to functionally validate the identified variant.

Results: Exome sequencing identified PIK3R1 c.1746-2A>C, a variant in the proband, father and paternal grandmother. The proband had neurodevelopmental delays, unilateral hearing loss and sensory processing disorder. His father has multiple cutaneous hemangioma, lipomas and early developmental delays that have since resolved. The paternal grandmother has macrocephaly. Its pathogenicity is supported by absence in population databases, position at a splice site acceptor junction, and abnormal splicing as demonstrated by RNA-seq. This variant has been reported once before in the mosaic state in an individual with overgrowth.

Conclusion: We report the first association of a rare germline variant in PIK3R1 with vascular malformations, overgrowth, and developmental delay, similar to activating PIK3R1 somatic variants. We will review these findings in the context of our expanded understanding of genotype-phenotype correlations with PIK3R1-related disorders.

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P06.050.A Clinical heterogeneity in *GNAS*-related disorders:experience of a single center

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Background: Pseudohypoparathyroidism (PHP), pseudopseudohypoparathyroidism (PPHP), and McCune-Albright syndrome (MAS) are disorders associated with mutations in the GNAS gene, which encodes the alpha subunit of the stimulatory G protein (Gsα). Due to the complex expression of the GNAS gene, these conditions present a broad clinical spectrum. Loss-of-function mutations cause PHP or PPHP phenotypes, characterized by hormone resistance or Albright hereditary osteodystrophy (AHO) features, respectively. Activating mutations lead to MAS with fibrous dysplasia and endocrine abnormalities. This study describes the phenotypic and molecular spectrum of six cases diagnosed with GNAS-related disorders.

Material and methods: Six Ege University Pediatric Genetics Department patients were selected. Hospital records were reviewed for clinical and molecular data.

Results: The study included six patients (4 females, 2 males) aged 5–16 years, presenting with seizures, developmental delay, short stature, obesity, hypocalcemia, and premature menarche. Physical examination revealed AHO features in four patients, café-au-lait spots in one, obesity in two, and intellectual disability in three. TSH resistance was observed in four cases, and PTH resistance in three. Molecular analysis identified pathogenic GNAS variants in five cases, four of which were novel. One patient also had a maternally inherited 22q11.2 duplication, explaining their dysmorphic features. For a patient with premature menarche and fibrous dysplasia, skin biopsy-based high-read-depth NGS was planned. Diagnoses included PHP type 1a (4 cases), PPHP (1 case), and MAS (1 case).

Conclusion: *GNAS*-related disorders demonstrate diverse clinical features influenced by mutation type and inheritance. Molecular studies from affected tissues are critical for diagnosing MAS cases with suspected mosaicism.

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P06.051.B X-chromosome inactivation patterns and clinical manifestations in Russian females with *EDA* mutations

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Background/Objective: X-linked hypohidrotic ectodermal dysplasia (XLHED) is a rare disorder affecting various ectodermal derivatives due to pathogenic variants in the EDA gene. Female carriers of EDA deleterious mutations typically exhibit milder or no clinical signs compared to affected males. This study aimed to determine whether a correlation exists between HED manifestations and X-chromosome inactivation (XCI) patterns.

Material and methods: XCI patterns were analyzed in the blood of 47 Russian female patients carrying pathogenic or likely pathogenic EDA variants using an assay that targets the polymorphic CAG repeats of the AR gene. Peripheral blood DNA was digested with the HpaII enzyme, which cleaves only the restriction sites on the active X chromosome, leaving the methylated X-chromosome intact. XCI patterns were classified as random if the ratio was ≤75:25, moderately skewed if between 75:25 and 80:20, and highly skewed if >80:20.

Results: The analysis of XCI patterns in 47 females with causative EDA variants revealed no significant differences between the individuals ($p = 0.277$), indicating no clear link between preferential XCI patterns and clinical XLHED manifestations. In most cases, blood X-inactivation was random. Notably, in one family, female subjects displayed almost opposite XCI patterns, yet both exhibited HED-related clinical features.

Conclusion: Our findings indicate no evidence of preferential X-inactivation in blood among female XLHED Russian patients and EDA female carriers. Consequently, blood X-inactivation testing should not be widely used in clinical practice for predicting XLHED severity or for refining the pathogenicity of variants of uncertain significance (VUS) in the EDA gene.

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P06.052.C A family with new X-linked overgrowth syndrome caused by *SPIN4* gene defect

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Background: Spindlin Family Member 4 (*SPIN4*) is an epigenetic reader, located on the X-chromosome and recently reported to negatively regulate body growth. Normally SPIN4 binds to modified histones, promotes canonical WNT signaling, and inhibits cell proliferation. The loss of SPIN4 function is associated with overgrowth syndrome in mice and humans, hemizygous males being more affected.

Material and Methods: 14-year-old male was assessed for suspected overgrowth syndrome. Within the family, next-generation sequencing (NGS), Sanger sequencing, familial segregation analysis, and skewed X-inactivation (androgen receptor (AR)-CAG trinucleotide repeat length) were used.

Results: The male proband presented tall stature (+3.5SD), thick joints, and normal intelligence. He was born large for gestational age with birth weight of +3SD and length of +2SD. At presentation, puberty was at Tanner stage 5 with testicular volume >25 ml. Ultrasound revealed splenomegaly. Bone age was consistent with the chronological age. Low bone mineral density was detected in the spine and the entire body (L1-L4 BMD HAZ −1.08 SD; TBLH BMD HAZ: −3.29 SD). Clinical biochemical evaluations (IGF-1, IGFBP-3, PTH, 25-OH vitamin D, Ca, P, Mg, thyroid function tests, and creatinine) were in the normal range.NGS identified a previously described hemizygous frameshift variant in the *SPIN4* gene (NM_001012968.3(SPIN4):c.312_313del p.(Arg104Serfs*24)). The variant was present in the proband's mother and maternal grandmother, although they both had skewed X-deviation (80% and 20%) and no height gain to their midparental height.

Conclusion: We report a second family with a newly identified X-linked overgrowth syndrome caused by a loss-of-function variant in the *SPIN4* gene.

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P06.053.D Identification of novel recurrent gross deletion in *COL9A3* gene in Russian patients with multiple epiphyseal dysplasia

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Background: Multiple epiphyseal dysplasia (MED) is a heterogeneous chondrodysplasia with autosomal dominant or recessive inheritance characterized by joint pain and stiffness, waddling gait and/or mild short stature in childhood. The disorder is associated with mutations in several genes, including *COL9A3*, which encodes one of the three alpha chains of Type IX collagen. Heterozygous variants in *COL9A3* have been implicated in MED pathogenesis.

Material and methods: Whole-genome sequencing (WGS) was performed to 6 families of Russian descent with MED using the DNBSEQ-T7 platform. The obtained data achieved an average coverage depth of 35×.

Results: WGS revealed a previously unknown gross deletion, chr20:62817899_62818755del, spanning 857 nucleotides length and including exon 3 of gene *COL9A3*. This deletion was detected in 18 patients with MED across all six families and segregated with the disease in each case. All families were of Russian origin but represented diverse regions within the country. Importantly, this variant has not been published before and not present in the population frequency databases, such as DGV Gold and gnomAD SV. Based on established criteria, the deletion was classified as a pathogenic variant.

Conclusion: Pathogenic variants affecting splicing and leading to skipping of exon 3 in patients with MED have been repeatedly reported in this gene, highlighting the functional importance of this region. The newly identified gross deletion in *COL9A3* gene represent a frequent recurrent pathogenic variant among Russian patients with MED. These findings expand the mutational spectrum of *COL9A3* and emphasize the need for targeted genetic screening in affected patients.

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P06.054.A Exploring *EDA*gene splicing variants: functional insights, clinical implications and therapeutic opportunities

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Background: X-linked hypohidrotic ectodermal dysplasia (XLHED), characterized by hypotrichosis, hypohidrosis, hypodontia, is caused by *EDA* (NM_001399.5) pathogenic variants, which alter ectodermal morphogenesis. To date, 416 *EDA* mutations have been reported and approximately 10% of them affects normal splicing process.

Material and methods: Whole exome sequencing (WES) was performed in one family, highly suggestive of XLHED. In vitro splicing assay using *EDA* minigenes was used to evaluate splicing variant effects.

Results: Our proband presented with hypodontia, sparse hair and dry skin, while his mother and sister showed milder XLHED phenotypes. In trio-WES analysis identified a hemizygous c.924+6T>G *EDA* variant in the proband, with the mother and the sister being heterozygous for the same variant. The variant, located near the splice donor site in intron 7, was predicted to be damaging by in silico tools. The minigene assay showed that mutant construct produced one correctly spliced transcript and a shorter one due to a cryptic donor splice site activation. This led to a 42 bp deletion in exon 7, causing a 14-amino-acid loss in the functional TNF domain.

Conclusion: This study underscores the importance of integrating WES data with functional assays to elucidate the role of splicing variants in *EDA* gene, especially those involving the exon 7 mutational hotspot. Functional validation provides critical insights into the XLHED's molecular basis and serves as a valuable tool for determining the significance of variants of unknown significance. Furthermore, promising outcomes from both preclinical and clinical studies on targeted therapeutic strategies for XLHED emphasize the need to improve advanced genomic approach.

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P06.055.B A de novo 1.7 Mb interstitial deletion upstream of *SOX9* in a patient with acampomelic form of campomelic dysplasia

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Background: Acampomelic campomelic dysplasia (ACD) is a rare autosomal dominant skeletal disorder classified as a milder form of campomelic dysplasia (CD). ACD typically results from heterozygous de novo pathogenic variants in *SOX9* and is characterized by distinct clinical features, including spinal malformations, cleft palate, facial dysmorphism, and an absence of long bone bowing, which differentiates it from classic CD. Approximately two-thirds of patients with ACD exhibit 46,XY sex reversal, often due to disruptions in *SOX9* regulatory elements. We report a case of ACD with 46,XY sex reversal caused by a de novo ~1.7 Mb interstitial deletion at chromosome 17q24.3.

Material and methods: High-resolution 1M array comparative genomic hybridization (aCGH) identified the chromosomal aberration, which was subsequently validated and refined using quantitative real-time polymerase chain reaction followed by Sanger sequencing to map the exact breakpoints.

Results: The analysis revealed a de novo 1.671 Mb deletion upstream of *SOX9* in a 3-year-old female patient with a 46,XY karyotype. The deleted region encompassed key *SOX9* enhancers, including F2, SOX9cre1, enh3, enh4, enh5, enh6, enh7, F8, E1, and E3. Clinically, the patient presented with a short neck, thoracic kyphoscoliosis, widely spaced nipples, sandal gap deformity, cleft palate, gonadal dysgenesis, and mild facial dysmorphism.

Conclusion: This case represents the second-largest deletion upstream of the *SOX9* gene associated with ACD reported to date. It underscores the role of *SOX9* enhancers in modulating the clinical variability of ACD and expands the spectrum of chromosomal abnormalities linked to *SOX9*-related disorders. Our findings provide valuable insights into genotype-phenotype correlations in ACD.

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P06.056.C Unveiling *IL11RA* variants in craniosynostosis and dental anomalies: insights from exome sequencing

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Background: Craniosynostosis (CS) is the second most common craniofacial birth defect after orofacial clefts, affecting approximately 1 in 2,250 live births. This condition results from the premature fusion of one or more cranial sutures, causing abnormal skull development. CS is genetically heterogeneous, presenting as either rare syndromic or more commonly nonsyndromic forms. Bi-allelic pathogenic variants encoding the interleukin 11 receptor alpha (*IL11RA*) gene have been implicated in CS and dental anomalies (CRSDA), a Crouzon-like craniosynostosis disorder.

Material and methods: Exome sequencing (ES) was conducted in two cases. Variants in genes associated with CS, based on the Human Phenotype Ontology and literature data, were analyzed. Rare variants' pathogenicity was assessed and segregations were performed using Sanger sequencing.

Results: Two cases presenting with craniosynostosis and dental anomalies were clinically evaluated. Additionally, one of the cases exhibited symptoms of epilepsy. Following the clinical assessments, ES was performed on both cases. ES identified two different homozygous variants in the *IL11RA*(NM_001142784). The first case revealed a previously reported nonsense variant (p.(Arg237*)), while the second case identified leading to a novel frameshift alteration (p.(His289Profs*70)). Both variants were confirmed as homozygous in the affected individuals, with their parents identified as heterozygous carriers.

Conclusion: Craniosynostosis is a genetically heterogenous condition with a complex etiology involving monogenic, polygenic, and chromosomal factors. This study underscores the value of ES in uncovering the genetic basis of CS. Identifying *IL11RA* variants in two CRSDA cases expands the spectrum of known pathogenic variants and enhances the understanding of the genetic mechanisms underlying the condition.

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P06.057.D A Novel pathogenic variant in the PDE4D gene in a case of metaphyseal acroscyphodysplasia

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Background: Metaphyseal acroscyphodysplasia (OMIM 250215) is an ultra-rare skeletal dysplasia classified within the group of metaphyseal dysplasias. Acroscyphodysplasia includes radiological findings, facial dysmorphism, obesity and mental retardation. This condition is also characterized by significant brachydactyly and the presence of cone-shaped epiphyses in the hands. To date, five clinical cases of metaphyseal acroscyphodysplasia caused by pathogenic variants in the *PDE4D* gene have been published.

Material and methods: The patient, a 7-year-old, underwent an extensive evaluation involving pedigree analysis, clinical and X-ray, Whole-genome sequencing, and direct Sanger sequencing to establish a precise diagnosis.

Results: We present a sixth clinical case of metaphyseal acroscyphodysplasia featuring a previously unreported pathogenic variant in the *PDE4D* gene: c.934C>T (p.Leu312Phe) in exon

7. A variant in the PDE4D gene characterized by an alternative amino acid substitution has been previously described in acrodysostosis type 2. The clinical characterization reveals distinct features of the facial phenotype, obesity, disproportionate short stature accompanied by limb shortening, pronounced brachydactyly affecting both hands and feet, as well as intellectual disability. Radiographic examinations of the upper and lower extremities reveal the presence of cone-shaped epiphyses accompanied by cup-shaped enlarged metaphyses in the knee regions, along with notable humeral bowing and brachydactyly with cone-shaped epiphyses.

Conclusion: The identification of a novel variant in the *PDE4D* gene expands the existing mutational spectrum and offers important insights for future investigations into the genotype-phenotype correlations associated with Metaphyseal acroscyphodysplasia.

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P06.058.A Report on a new splice variant in *ACAN* in a well-characterized family

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Background: Spondyloepiphyseal dysplasia is a rare disorder caused by variants in several genes; among others, more than 100 different alterations in the ACAN gene (missense, nonsense and splice variants) have been identified as causative. In general, identification of new splice variants is often complex, as the effect of a variant in the exon/intron transition is difficult to predict. Therefore, the family presented here, in which a causal splice alteration can be segregated over several generations with a clear phenotype, is helpful.

Material and methods: The reported family introduced themselves due to the short stature of the youngest daughter (p3). The initial diagnosis was conducted using a multigene panel in our DAkkS accredited laboratory. Subsequently, the identified variant was segregated in the rest of the family. Additionally, a transcriptome analysis was performed.

Results: In the index patient, a novel variant was identified in the intron of the 5' UTR (NM_001369268.1: c.-7-1G>T), which is shared by sister and mother. The clinical-genetic examination of the extended family revealed that the sister and the mother of the index were also affected by spondyloepiphyseal dysplasia; in addition to short stature. Both suffer from osteochondrosis dissecans. We expect that the transcriptome analysis, which is still pending, will provide further insights into the functional mechanisms of the variant.

Conclusion: The symptoms of several affected individuals in combination with the segregation results allow the variant to be assessed as probably pathogenic and expands the group of known disease-causing variants and diagnostics.

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P06.059.B Expanding the phenotypic and genetic spectrum of *PIEZO2*-related distal artrogryposis type 5: a single center experience

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Background: Distal arthrogryposis type 5 (DA5) is a rare autosomal dominant *PIEZO2*-related disease, characterized by congenital contractures, ophthalmoparesis, ptosis and restrictive respiratory disorders. We studied the phenotypic manifestations of 9 patients from 8 unrelated families with DA5 caused by four previously described and one novel heterozygous nucleotide variants in the *PIEZO2* gene.

Material and methods: All patients underwent comprehensive clinical, radiological examinations and electromyography study. Trio WGS was conducted for three families, and WES for 5 patients. Almost all cases (7 families) have de novo status, which was confirmed by Sanger sequencing.

Results: The most severe clinical manifestations with respiratory failure and tracheal stenosis were found in patients with novel variant c.8238G>A (p.Trp274Ter) and another c.7095G>T (p.Trp2365Cys), which hadn't been revealed in heterozygous state. While in patients with other previously described variants c.8181_8183delAGA (p.Glu2727del), c.2134A>G (p.Met712Val) and c.8555C>T (p.Ser2852Leu) the clinical phenotype was more moderately expressed. Our cohort included patients with different ages (from 1 to 24 years) to successfully identify a number of previously not reported symptoms of DA5, such as uneven distribution and thickening of subcutaneous fat in the abdomen and thighs, poor temporal hair growth, elongated second toe and short first toe, as well as to assess the formation of the disease phenotype in dynamics.

Conclusion: It has been shown that spectrum of DA5 clinical manifestations depends on the localization of amino acid substitution in a protein molecule and the disease progression may occur as the child grows, which requires monitoring of this group of patients.

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P06.062.A A Novel SOX9 variant leading to Mild campomelic dysplasia

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Background: Campomelic Dysplasia is a congenital genetic disorder caused by mutations in the SOX9 gene, characterized by skeletal deformities, respiratory distress, and other systemic features. This disorder typically manifests with growth and developmental delay and facial dysmorphisms. We aim to present a novel case of midly affected Campomelic Dysplasia expanding the clinical spectrum.

Material and methods: The patient presented with respiratory distress at birth, requiring intubation for one month and then tracheostomy at 3 months of age. At the age of two years, the patient developed gait abnormalities, leg length discrepancy and knee deformities and was referred to us. A multigene panel test was performed.

Results: On physical examination, the patient exhibited macrocephaly, a long triangular face, wide forehead, periorbital edema, scoliosis, pes planus and short fingers. X-ray radiography revealed scoliosis and metaphyseal enlargement of long bones

and flat acetabular roof. We identified a heterozygous de novo novel variant in the SOX9 gene (c.440_445del). These findings, combined with the clinical presentation, led to the diagnosis of Campomelic Dysplasia.

Conclusion: We consider this case as a mild campomelic dysplasia due to milder skeletal involvement and missing genitourinary abnormalities. Early identification through NGS allows for accurate diagnosis and better management in such mild phenotypes. Genetic counseling plays a key role in guiding affected families.

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E. Yilmaz Gulec: None. **B. Adacoglu:** None.

P06.063.B Expanding phenotype Of oral-facial-digital syndrome caused by a homozygous putative pathogenic variant in the *INTU* Gene

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Background/objectives: To date, more than 15 genetic causes of oral-facial-digital (OFD) syndrome have been described. This syndrome is associated with abnormalities of the face (hypertelorism), oral cavity (lingual hamartoma) and extremities (polydactyly). Renal and cerebral malformations are also associated with OFD. OFD syndrome belongs to the ciliopathies. In 2017, Bruel et al. reported the first patient with OFD with a homozygous pathogenic variant in *INTU*. To date, few pathogenic variants in *INTU* are known.

Methods: We report a newborn boy of consanguineous parents with facial features including hypertelorism, broad nasal root and malformation of the extremities consisting of pre- and postaxial hexadactyly and syndactyly. He had respiratory difficulties due to laryngeal stenosis (retrolaryngeal mass, notched epiglottis, severe laryngomalacia). Laser supraepiglottoplasty of the large retrolaryngeal space-occupying lesion (plus tissue) was performed to avoid aspiration of the arytenoid cartilage and epiglottis. A tracheostomy tube was required to stabilise the patient due to vocal cord paresis.

Results: On suspicion of OFD syndrome, trio exome sequencing revealed a homozygous putative pathogenic variant (NM_015693.4:c.2428G>C p.(Gly810Arg)) in the gene *INTU* in the newborn. Both parents were heterozygous carriers of this variant. The variant is predicted to be protein-damaging according to prediction tools (e.g. CADD score 27.4).

Conclusion: A systematic review of the literature showed that only a few patients with putative pathogenic variants in *INTU* are known. The patient's phenotype overlaps with the clinical features reported in the literature. Severe upper airway obstruction with laryngomalacia and rigid vocal cord paresis is a previously undescribed association.

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P06.065.D Twelve years to diagnosis: *MCRS1*as a cause for Klippel-Feil syndrome

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Background: MCRS1 is a multifunctional protein essential for cellular processes. Research has shown that it is required for branchial arch and cranial cartilage development. Notably, Mcrs1 functionally interacts with Six1 in the embryo, playing a critical role in craniofacial tissue development, including the formation of the otic vesicle. Klippel-Feil syndrome is a congenital disorder characterized by fusion of cervical vertebrae, often accompanied by a short neck, scoliosis, hearing loss, and other skeletal abnormalities. We hypothesize that variants in *MCRS1* could be an additional etiologic factor for the syndrome.

Material and methods: Trio-genome and RNA sequencing from skin fibroblasts were performed. Identified variant was validated by Sanger sequencing.

Results: We report a 13-year-old boy with a potentially deleterious heterozygous 406 bp de novo deletion in *MCRS1* gene. His features include a short neck, dysmorphic facial features, missing ribs, spine abnormalities (including scoliosis), congenital hearing loss, bilateral *n. vestibulocochlearis* aplasia, and microcephaly. He has a clinical diagnosis of Klippel-Feil syndrome but no molecular diagnosis was identified until recently. Underexpression of the *MSCRS1* gene was found in RNA sequencing analysis, corresponding to a heterozygous loss-of-function defect. Reanalysis of genome sequencing data revealed a deletion in *MCRS1*.

Conclusion: We describe a patient with a clinical diagnosis of Klippel-Feil syndrome and a deletion in *MCRS1*. Given the gene's important role in craniofacial and branchial arch development, we hypothesize it could be the cause for the patient's clinical picture. We are planning to conduct additional studies in order to determine the functional impact of the variant.

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E. Tiivoja: None. **R. Teek:** None. **S. Pajusalu:** None. **K.T. Oja:** None. **M.H. Wojcik:** None. **I. Osei-Owusu:** None. **K. Ounap:** None.

P06.066.A When icthyosis reveals a rare metabolic disorder: a case of Chanarin-Dorfman syndrome

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Background: Chanarin-Dorfman syndrome (CDS) is a rare autosomal recessive disorder caused by mutations in the ABHD5 gene, leading to intracellular triacylglycerol accumulation in various organs. It presents with ichthyosis, multisystem involvement, and lipid-filled vacuoles in neutrophils, known as Jordan's anomaly.

Material and Methods: A 19-month-old female, born of a third-degree consanguineous marriage, presented with xerotic, scaly skin since birth, progressive abdominal distension, and hepatosplenomegaly. Clinical examination revealed lamellar scales, ectropion, dysplastic auricles, and hypohidrosis. Laboratory investigations indicated elevated transaminases, hypertriglyceridemia, and lipid vacuoles in neutrophils. Genetic confirmation through whole-exome sequencing identified a homozygous pathogenic variant in the ABHD5 gene [c.810T>A: p.(Tyr270*)].

Results: The diagnosis of CDS was confirmed. Multidisciplinary management, including dietary fat restriction, medium-chain triglyceride supplementation, skincare, and supplementation with ursodeoxycholic acid and vitamin E, improved growth, metabolic markers, and imaging findings. Follow-up showed reduced AST and ALT levels, lower triglycerides, and decreased liver size.

Conclusion: This case highlights the characteristic clinical, laboratory, and genetic findings in CDS, emphasizing the

importance of a multidisciplinary approach to management. Early diagnosis and intervention improve outcomes, while genetic counseling and prenatal diagnostic testing are essential to prevent recurrence in future pregnanciesFigure 1: Peripheral smear analysis demonstrating cytoplasmic vacuolation of neutrophils and eosinophils.Figure 2: Finer, grayish, semi-adherent scales on the trunk

C.O.I. Disclosure:

D. Singh: None. **D. Kaur:** None. **P. Panigrahi:** None. **D. Mahajan:** None. **D. Bhatia:** None.

P06.067.B Recurrent FGFR3 splicing variant in a case of atypical achondroplasia: a case report

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Background: Pathogenic variants in *FGFR3* gene are associated with a broad range of skeletal dysplasias, ranging from achondroplasia to thanatophoric dysplasia. Despite highly suggestive clinical features, some cases lack a definitive molecular diagnosis, with routine diagnostic testing failing to identify a pathogenic variant.

Material and methods: Clinical assessment of a patient with a de novo *FGFR3* variant presenting with a clinical phenotype compatible with an atypical form of achondroplasia.

Results: An 8 year-old girl patient presented with disproportionate short stature, rhizomelic shortnening of the limbs, *genu varum*, and lumbar hyperlordosis. Cognitive development was adequate for her age, whereas a mild gross motor delay was described. No particular dysmorphic features were present; a relative macrocephaly was described. Radiographic features were highly suggestive for *FGFR3* spectrum. Prior sequencing of *FGFR3* (performed on both blood and buccal swab samples), NGS-based gene panel for skeletal dysplasias, Whole Exome Sequencing, and exome-based arrayCGH failed to identify any pathogenic variant. Whole Genome Sequencing identified a pathogenic intronic heterozygous de novo variant in *FGFR3* (*FGFR3*:c.1075+95C>G), previously reported in three patients with atypical achondroplasia (or severe hypochondroplasia), and one patient with hypochondroplasia.

Conclusion: We identified the *FGFR3*:c.1075+95C>G variant in a girl presenting with disproportionate short stature and typical skeletal phenotype of *FGFR3* spectrum. Consistently with previous reports, the variant appears to be associated with an atypical form of achondroplasia or severe hypochondroplasia. Our results provide an additional pediatric report of the clinical phenotype associated with this recurrent splicing variant, reaffirming the necessity to include it in diagnostic setting.

C.O.I. Disclosure:

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P06.070.A Long-term impact of spondylometaphyseal dysplasia, corner fracture type (SMD-CF) caused by heterozygous pathogenic variant in *FN1* gene

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Background: Spondylometaphyseal dysplasia, corner fracture type (SMD-CF; OMIM#184255, ORPHA:93315) is a rare skeletal dysplasia presenting with short stature, developmental changes in vertebrae, corner fracture-like lesions and metaphyseal abnormalities with sparing of the epiphyses. The disease has large phenotypic diversity and identified causative genes are *COL2A1* and more recently discovered *FN1*. Less than 20 cases of *FN1* associated SMD-CF have been described in the literature. We seek to improve understanding of SMD-CF long term course by retrospectively analyzing one patient's lifetime medical data.

Material and methods: We conducted a retrospective study on the medical data, including radiological images, of a 54-year-old patient with clinical suspicion of SMD-CF and assessed the clinical phenotype at the age of 53. Molecular diagnosis was confirmed by next generation sequencing (NGS).

Results: Most notable findings included short stature (−3.5SD), short trunk, waddling gait, scoliosis, *pectus excavatum*, F2-5 clinodactyly, left foot T2-3 clinodactyly, flat feet, overpronating knees, myopia and optic disc drusen associated with visual field defect. Radiological images revealed corner fractures present since childhood and progressive osteoarthritis, ultimately leading to the endoprostheses of two joints (right knee at 52y, and left hip at 49y). NGS analysis detected a heterozygous pathogenic variant in the *FN1* gene; NM_212482.3(FN1):c.693C>G p.(Cys231Trp).

Conclusion: Our study demonstrates the long-term course of SMD-CF and provides valuable information to clinicians regarding prevention and treatment, as well as to patients and their families regarding prognosis. Since *FN1* pathogenic variants in SMD-CF patients have been rarely described, this study offers invaluable insight into the phenotype-genotype correlation.

C.O.I. Disclosure:

E. Tars-Hurt: None. **M. Lintrop:** None. **R. Zordania:** None. **L. Mauring:** None. **K. Ounap:** None.

P06.071.B Atypical glutamic acid to lysine substitution in the triple helix of type III collagen manifests as overlap between classical and vascular Ehlers-Danlos syndrome

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Background: Vascular Ehlers-Danlos syndrome (vEDS) is caused by pathogenic variants in *COL3A1*. The most frequently reported pathogenic variants are glycine substitutions within the triple-helical domain of type III procollagen and splice site variants leading to an exon skip. A 34-year-old man was referred because of suspected connective tissue disorder. At birth, bilateral congenital clubfeet were observed. Joint hypermobility was present in childhood, and recurrent shoulder dislocations began at the age of 29 after a fall. There was a history of delayed wound healing and wound dehiscence, but no signs suggestive for vascular fragility. Clinical examination showed hyperelastic, translucent, doughy skin, generalized joint hypermobility (Beighton score 9/9), facial features (hypotelorism, deep-set eyes and epicanthal folds), a flattened back and mild pedes plani. An

atrophic scar with hemosiderin deposition was observed on his left leg.

Methods and materials: Due to the combination of skin fragility and joint hypermobility, classical EDS was suspected. Whole exome sequencing of known EDS-associated genes was performed.

Results: A variant of unknown significance in *COL3A1* in the triple helix (p.(Glu730Lys)) was identified. Segregation analysis revealed the variant in the younger brother (33y), with a comparable phenotype. Hence the variant was reclassified as likely pathogenic.

Conclusion: We describe two patients with a novel glutamic acid-to-lysine substitution in *COL3A1*, currently without complications related to tissue fragility. The clinical presentation of our patient shows overlap with previously reported cases, often suspected as classical EDS, with some exhibiting vEDS-like features. Additional cases are needed to clarify complication risks and the underlying pathophysiology.

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P06.073.D Gonadal mosaicism as a rare inheritance pattern in recessive genodermatoses: report of two cases with Pseudoxanthoma Elasticum and literature review

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Background: Gonadal mosaicism, the presence of genetically distinct cell populations in the gonads, is well-documented in autosomal dominant genodermatoses but rarely reported in autosomal recessive diseases. We describe two families with Pseudoxanthoma Elasticum (PXE), an autosomal recessive genodermatosis caused by biallelic ABCC6 mutations, and systematically review gonadal mosaicism in recessive skin disorders.

Material and methods: Genetic analyses, including direct sequencing and MPLA, were conducted on both families. A systematic literature review was performed following PRISMA-P guidelines.

Results: The first male proband, diagnosed with PXE at age 11, exhibited characteristic skin and ocular findings and was compound heterozygous for the c.3506 + 2T > C variant (maternal) and a whole ABCC6 gene deletion (uncertain paternal origin). The second female proband, diagnosed at 16, had typical PXE features and was compound heterozygous for p.R1141* (maternal) and a whole ABCC6 deletion, with evidence suggesting paternal germline mosaicism. Asymptomatic family members carried one pathogenic variant, and analyses ruled out UPD16, high-level mosaicism, and non-paternity. The literature review identified 16 additional gonadal mosaicism cases in recessive skin disorders, including cutis laxa, epidermolysis bullosa, Cockayne syndrome, and Vici syndrome, with paternal mosaicism more frequent.

Conclusion: Recognizing gonadal mosaicism is crucial for genetic counseling, as recurrence risk may be significantly lower than the typical 25%, impacting reproductive planning and genetic testing accuracy. Expanded carrier screening may overlook gonadal mosaicism, leading to misinterpretations. These findings highlight gonadal mosaicism as a possible inheritance mechanism in PXE and emphasize the need for expert interpretation of genetic results.

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P06.074.A A novel pathogenic variant in FBN1 gene identified in a patient with MFS

Susanna Gastaldi[1], *Miriam Carriero*[1], *Monia Magliozzi*[2], *Antonio Novelli*[3], *Federica Sangiuolo*[1,4], *Leila Baghernajad Salehi*[1], *Giuseppe Novelli*[1,4]

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Introduction: Mutations in the fibrillin-1 (FBN1) gene are linked to Marfan syndrome (MFS) and MFS-related disorders (MFS-RD), which exhibit a range of overlapping phenotypes. MFS is an autosomal dominant disorder affecting connective tissue, primarily in the heart, eyes, and skeleton. Despite its well-established clinical features, factors influencing phenotypic severity remain unclear. Molecular analysis of the FBN1 gene, located on chromosome 15 and containing 65 coding exons, helps reduce diagnostic uncertainty in MFS and MFS-RD. To date, more than 2700 mutations in FBN1 have been documented, with up to 25% of cases arising from de novo variants.

Case Report: We describe a 53-year-old man diagnosed with congestive heart failure at 32, secondary to severe mitral insufficiency from mitral valve prolapse. He underwent mitral and tricuspid valvuloplasty and was later diagnosed with aortic bulb dilatation. His physical exam revealed a height of 188 cm, arm span of 210 cm, small joint hyperextensibility, positive bilateral thumb and wrist signs, arachnodactyly, and scoliosis with right convexity.

Discussion: Informed consensus was obtained from patient and Next Generation Sequencing analysis was performed on patient's peripheral blood sample. A novel heterozygous FBN1 variant, c.6865T>A, resulting in a cysteine-to-arginine substitution (p.Cys2289Arg), was identified. The identified variant is a novel VP, not so far been reported in the scientific literature in individuals with Marfan syndrome.

Conclusions: Present work enriches the mutations profile in FBN1 gene associated with MFS and underlines the importance of updating clinical criteria considering the complexity of cardiological clinical status.

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P07 Cardiovascular disorders

P07.001.B Generation and characterization of two novel knock-in models: insights into arrhythmogenic cardiomyopathy

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Guglielmini[1], Paola Braghetta[1], Libero Vitiello[1], Alessandra Rampazzo[1], Martina Calore[3]

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Background: Arrhythmogenic cardiomyopathy (ACM) is a genetic disorder characterized by nonischemic ventricular arrhythmias and sudden death. ACM is generally caused by pathogenic variants in genes encoding for proteins of intercalated discs and it is defined by a progressive myocardial dystrophy resulting in fibro-adipogenic substitution.

Material and methods: To investigate the molecular mechanisms underlying ACM, two novel murine knock-in models were generated harboring the p.G105R mutation in the *Dsg2* gene (DSG2_KI) and the p.del765L in-frame deletion in the *Ctnna3* gene (CTNNA3_KI), in both homozygous and heterozygous forms. The mice were analyzed at ultrastructural, molecular and functional level. Specifically, transmission electron microscopy images were analyzed, real-time PCR, echocardiography and electrocardiogram (ECG) were performed.

Results: Knock-in models displayed a reduction of desmosome length at different ages; in particular, in homozygous DSG2_KI animals desmosomes were even shorter compared to the heterozygous ones. Moreover, homozygous DSG2_KI mice also exhibited a greater expression of transcripts associated to fibrosis. The same increase in fibrosis markers were observed in the heterozygous CTNNA3_KI model. Functionally, in 9-month-old heterozygous DSG2_KI mice, fractional shortening, ejection fraction and left ventricular internal diameter at end-diastole (LVIDd) were elevated, while left ventricular internal diameter at end-systole (LVIDs) was decreased. Finally, by means of ECG, extrasystole events were observed in 12-month-old heterozygous animals.

Conclusion: Altogether, these results suggest that the two knock-in lines exhibited typical features of ACM, such as shorter desmosomes, fibrosis and functional alterations. Therefore, these models provide valuable tools to explore the molecular mechanism behind the disease and explore potential therapeutic interventions.

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P07.002.C DNA methylation in *ABCG1* mediates an effect of lipoproteins on atherosclerotic markers

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Background: Atherosclerosis, linked to cardiovascular disorders like myocardial infarction and stroke, involves chronic low-grade inflammation, though its mechanisms remain unclear. This study explores the role of blood lipids and DNA methylation in atherosclerosis and its influence on cardiovascular outcomes.

Material and methods: We analyzed 112 serum NMR lipoprotein subfractions in 4,010 individuals from the SHIP-TREND cohort, examining associations with subclinical atherosclerosis markers (e.g., carotid intima-media thickness, periodontitis, retinal microcirculation, and inflammatory cytokines). An epigenome-wide association study (EWAS) was conducted in 479 individuals using the EPIC array to identify CpG sites linked to lipoproteins and atherosclerosis. We tested the association of DNA methylation levels of the significant CpG sites with atherosclerotic markers and cardiovascular outcomes.

Results: There were significant associations between lipoproteins and atherosclerotic traits, particularly low-density lipoproteins (LDL) and inflammatory markers, potentially mediated by DNA methylation. Two CpG sites, cg05325763 (chromosome 11) and cg06500161 (chromosome 21 in *ABCG1*), were strongly associated with LDL ($p < 4.73E\text{-}10$). Methylation at cg06500161 correlated with increased soluble tumor necrosis factor receptor 2 (sTNFr2) levels ($p = 0.024$) and myocardial infarction risk, while cg05325763 methylation was linked to reduced IL-10 levels ($p = 0.044$). Both gene expression and DNA methylation levels at *ABCG1* showed association signals with myocardial infarction occurrence in the analyzed population, supporting its role in cardiovascular outcomes.

Conclusion: These findings highlight the interplay between lipoproteins, DNA methylation, and inflammation in atherosclerosis, suggesting *ABCG1*'s potential role in myocardial infarction risk. This study advances understanding of atherosclerosis' inflammatory processes and its epigenetic regulation.

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P07.003.D LncRNA H19: promoter and circulating biomarker of human carotid atherosclerotic plaque development

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Background: Long non-coding RNAs (lncRNAs) have been increasingly involved in several diseases, including atherosclerosis; however, their specific role in plaque development is still under investigation. This study aimed to investigate the involvement of lncRNAs as potential effectors and biomarkers of carotid atherosclerosis in humans.

Methods: Carotid atherosclerotic plaques, their adjacent regions (AR) and plasma samples were collected from 15 patients and analyzed via total RNA-Seq by Next Generation Sequencing. Bioinformatic analysis was performed on R. Additionally, plasma samples from other 35 atherosclerotic patients and 19 healthy controls (50 cases, 19 controls) were collected. Circulating RNA was extracted and quantitative real-time PCR (qRT-PCR) was performed using SYBR Green, with GAPDH as housekeeping gene. Statistical analysis was performed using SPSS software.

Results: Among the 915 differentially expressed genes in plaques versus AR, lncRNA H19 was significantly overexpressed in plaques ($logFC = 4.3, p = 0.00004$) and detected in plasma. qRT-PCR confirmed H19 upregulation in plaques [16.5(6.5–40.5)] versus AR [0.1(0.07–1.8), $p = 0.001$]. Circulating H19 levels were higher in atherosclerotic patients [1.15(0.7–2.43)] than in controls

[0.7(0.3–1),p = 0.004] and were associated with lipid-rich plaques (Gray-Weale grade 2,p = 0.022), smoking (p = 0.028), and dyslipidemia (p = 0.020). Gray-Weale resulted independently negatively associated to H19 levels (beta-coeff.=−0.498 - p = 0.004).

Conclusion: Our findings suggest that lncRNA H19 is overexpressed in human carotid atherosclerotic plaques rather than in the adjacent lower-grade regions, confirming its implication in atherosclerotic plaques development in humans. Higher levels of H19 were found in plasma from atherosclerotic patients, associated with plaque instability, thus supporting its potential as a circulating biomarker (AUC = 0.73) for unstable carotid atherosclerosis.

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P07.004.A Genetic crossroads of cardiovascular disease and its comorbidities

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Background: With rising life expectancy, the prevalence of cardiovascular disease (CVD) patients with comorbidities is also increasing, complicating healthcare and outcomes. This highlights the need for improved prevention, treatment and management strategies. Understanding the genetic links between CVD and its comorbidities could advance research in this area. We aimed to assess the genetic correlation (r_g) between CVD and its comorbidities.

Material and methods: We used FinnGen R12 GWAS summary statistics for 24 CVD and its comorbidities related endpoints. Quality control steps in the GWAS involved exclusion of participants with ambiguous gender, high genotype missingness (>5%), excess heterozygosity (+-4SD) and non-Finnish ancestry. SNPs with high missingness (>2%), low HWE p-value (<1e-6) and low minor allele count (MAC < 3) were excluded. We estimated heritability for each endpoint and assessed the genetic correlations between them using linkage disequilibrium score regression (LDSC). Disease endpoints with heritability z-score below 4 were excluded because genetic correlation estimates for these diseases are generally noise and therefore unreliable, leaving 19 diseases for analysis. Genetic correlations with Bonferroni-adjusted p-values (adj.pval) < 0.05 were considered significant.

Results: Out of the 19 diseases, five CVDs (coronary atherosclerosis, transient ischemic attack, atrial fibrillation, myocardial infarction and heart failure) and seven comorbidities (type 2 diabetes, asthma, obesity, depression, chronic obstructive pulmonary disease, gingivitis and hypertension) showed significant genetic correlations (adj.pval < 0.05).

Conclusion: We found significant genetic correlations between CVD and its comorbidities, suggesting shared genetic variants. The findings may aid in developing joint risk stratification tools for CVD and comorbidities.

C.O.I. Disclosure:

P.P. Mishra: None. **B. Hamal Mishra:** None.

P07.006.C Genetic findings in a cohort of over 2300 individuals with clinically suspected heritable thoracic aortic disease

Emma Mårtensson, *Julie Hathaway, Johanna Huusko, Saija Ahonen, Johanna Tommiska, Kimberly Gall, Janica Djupsjöbacka, Mikko Muona, Inka Saarinen, Eija Seppälä, Tiia Kangas-Kontio, Lotta Koskinen, Pertteli Salmenperä, Samuel Myllykangas, Juha Koskenvuo*

Blueprint Genetics, Espoo, Finland.

Background: Heritable thoracic aortic disease (HTAD) is associated with high morbidity and mortality; sudden death due to aortic dissection may be the first presenting feature. Establishing a molecular genetic cause of HTAD allows for management based on the causative gene and identifying at-risk family members. In this study, we reviewed the genetic findings in a large cohort of individuals with suspected HTAD to assess the value of genetic testing in this population.

Material and methods: A total of 2,358 individuals were tested with an NGS panel including up to 53 genes associated with HTAD. Gene content varied due to the addition of genes to the panel over time. Sequencing and copy number variant (CNV) interpretation was performed following ACMG guidelines.

Results: Pathogenic (P) or likely pathogenic (LP) variants consistent with the individual's phenotype and disease inheritance were identified in 280 (11.9%) cases. The yield of testing was similar in children (12.8%[36/281]) and adults (11.7%[244/2,077] but was higher in deceased individuals (23.5%[8/34]). A total of 284 LP/P variants were identified; 19 of these were CNVs. 97.9% of LP/P variants (278/284) identified were in genes associated with extracardiac features in addition to HTAD. The most common diagnostic finding was an LP/P sequence variant in *FBN1*, and the most common LP/P CNV was a duplication involving the *ABCC6* and *MYH11* genes.

Conclusion: This work further demonstrates the importance of comprehensive genetic testing in individuals with suspected HTAD, especially in those with relevant findings on autopsy, to inform management for at-risk family members.

C.O.I. Disclosure:

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P07.008.A Mitral valve prolapse caused by TLL1 gain-of-function mutation

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Background: Mitral valve prolapse (MVP) is a common cardiac valvular anomaly that can be caused by mutations in genes of various biological pathways. We set out to identify the molecular basis of apparently dominant heredity of isolated MVP in a three generation kindred.

Material and methods: Clinical evaluation and echocardiography for all complying family members (n = 13). Whole exome and genome sequencing of two affected individuals, delineating shared heterozygous variants, further tested for segregation within the kindred. Wild type versus mutant Tolloid Like 1 (TLL1) enzymatic activity assays in media of transfected HEK293 cells.

Results: The only heterozygous variant segregating in the affected kindred as expected for dominant heredity of MVP was p.T253A, within the catalytic domain of TLL1. Of eight heterozygotes, six had MVP and two had trivial mitral regurgitation. Activity assay in extracellular media of HEK293-transfected cells demonstrated that over time, the enzymatic activity of the mutated TLL1 protein was ~3-fold higher than that of the wildtype.

Conclusion: *TLL1* encodes an extracellular metalloprotease regulating ECM composition and maintenance. Heterozygous Loss-of-Function *TLL1* mutations have been previously shown to cause autosomal dominant atrial septal defects. We now show through genetic and biochemical studies that a TLL1 Gain-of-Function mutation, prolonging the half-life of TLL1 active protein in the extracellular matrix (ECM), causes a different cardiac phenotype: autosomal dominant MVP with variable expressivity. Our findings enable novel insights into molecular pathways of valvular physiology and disease, and the differing phenotypes of Loss-of-Function and Gain-of-Function mutations of the same gene.

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P07.010.C Single nucleus transcriptomic profiling of cardiac cells in a novel transgenic mouse model of arrhythmogenic cardiomyopathy

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Background: Arrhythmogenic cardiomyopathy (ACM) is one of the most commonly inherited cardiomyopathies, characterized by the progressive substitution of the myocardium with fibrofatty tissue. Clinically, ACM is characterized by ventricular arrhythmias, syncope, and sudden cardiac death and shows wide phenotypic heterogeneity. Approximately half of ACM cases are attributed to inherited mutations in one or more genes encoding cardiac intercalated disc components. Among them, those encoding for plakophilin-2 (*PKP2*), desmoplakin (*DSP*), and desmoglein-2 (*DSG2*) are most commonly mutated.

Material and methods: In order to study the pathogenic mechanisms underlying the disease, we generated transgenic mice with a cardiomyocyte-specific overexpression of human *DSG2* carrying the p.G100R mutation (Tg-hG) found in an affected patient. To establish the transcriptomic ACM signature, we performed bulk and single nucleus-RNA seq on cardiac samples from 6 month-old Tg-hG and control mice.

Results: Tg-hG mice presented several clinical features of ACM, such as fibrous replacement, increased inflammatory markers, and cardiac dysfunction. Accordingly, enrichment analysis and network construction performed on bulk RNA seq data identified upregulation of immune- and extracellular matrix-related processes. Moreover, single nucleus-RNA seq analysis dissected the cellular composition of wild-type and transgenic hearts. In agreement with phenotypic data and bulk RNA-seq, interactome analyses highlighted the involvement of fibroblast-secreted factors in three key ACM-related networks: extracellular matrix accumulation, inflammation, and Wnt signaling.

Conclusion: Collectively, these findings identified numerous processes and cell populations potentially altered in Tg-hG mice that could shed light on ACM pathogenic mechanisms.

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P07.011.D Targeted mRNA sequencing improves classification of splicing variants in hypertrophic cardiomyopathies

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Background: Hypertrophic cardiomyopathies (HCM) are inherited cardiac diseases with an autosomal dominant transmission, among which MYBPC3 is the main causal gene responsible for haplo-insufficiency. RNA splicing variants in MYBPC3 appears to be a prevalent cause of HCM, however RNA analysis is not developed for these diseases because of cardiac tissue unavailability. Thus, these variants are often classified as Variant of Unknown Significance (VUS) and can't be use for clinical purposes. We propose an enrichment method to detect splicing aberrations in MYBPC3 causing cardiomyopathies from blood cells mRNA.

Material and methods: We selected 26 variants (16 intronics and 10 exonics) detected on DNA potentially affecting splicing. polyA+ RNA purified from venous blood cells were retro-transcribed, captured with optimized design and sequenced on NextSeq550. A specific bio-informatic pipeline was developed to automatically detect splicing events.

Results: The gene *MYBPC3* was very well covered and interpretable (RPM~6809, Mean depth~2947X). We detected a splice aberration for 19/26 (73%) of cases, consistent with their respective predictive score, among which 6 (32%) creates a novel junction; 8 (42%) modifies the proportional usage of annotated junctions and 5 (26%) leads to the retention of the entire intron. Then, we performed bio-informatic screening that was able to detect all pathogenic events, including intron retention even without abnormal junction.

Conclusion: Targeted mRNA sequencing from blood cells helps to classify splice affecting variants, thus improving the yield of molecular diagnostic. This method, associated with a specific bio-informatic pipeline can be used as a screening approach expanded to other cardiomyopathy genes.

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P07.012.A Unravelling shared pathomechanisms in syndromic thoracic aortic aneurysm disorders using single-cell RNA sequencing

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Background: Thoracic aortic aneurysm (TAA) is characterized by progressive enlargement of the aorta, increasing the risk of dissection. Current pharmacological treatments are limited and patients often require high-risk surgical procedures. This highlights the need for better understanding of the disease mechanisms to develop new therapies to stop or reverse TAA.

Material and methods: We analysed single-cell RNA sequencing data from aortic samples (ascending aorta and root) of the TAA mouse models C57Bl6J *Fbn1*$^{C1041G/+}$ and C57Bl6N *Ipo8*$^{-/-}$, and their respective wild-type (WT) littermates to identify convergent mechanisms. Samples were collected before and after aneurysm development to distinguish driving from compensatory mechanisms.

Results: Prior to the aneurysm formation, a few top shared DEGs were identified in vascular smooth muscle cells (VSMCs), including *Tnc, Sncg, Wif1, Rbp4*, and *Smad6*, and both models showed downregulation of the "Signaling by TGF-β family members" pathway. When the aneurysm was present, enrichment analysis in the VSMC cluster of both models revealed the involvement of focal adhesion, extracellular matrix (ECM), and cytoskeleton pathways, pointing towards mechanotransduction dysregulation. Additionally, we pinpointed a shared VSMC subcluster enriched in aneurysmal aortas, which showed a greater upregulation of ECM and focal adhesion genes (*Tnc, Fn1, Itga5*), along with higher expression of TGF-β-related genes (*Bmp4*, *Tgfb1*, *Tgfb2, Serpine1*) and lower expression of contractile genes (*Myh11*, *Cnn1*).

Conclusion: Dysregulation of mechanotransduction may be an important shared pathomechanism in *FBN1*- and *IPO8*-related TAA. Furthermore, a subset of VSMCs with a more extreme phenotypic switch might be involved in the pathogenesis of both TAA conditions.

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P07.013.B Trait based partitioned polygenic risk scores for biologically informed risk stratification of common cardiovascular diseases

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Background: Cardiovascular diseases (CVDs) remain a major global health burden. Coronary artery disease (CAD), stroke, heart failure (HF), peripheral arterial disease (PAD), and atrial fibrillation (Afib) are among the most common CVDs, exhibiting both shared and distinct genetic and biological components. This study aims to elucidate these genetic mechanisms and their potential to predict CVD subtypes using partitioned polygenic risk scores (PRS).

Material and methods: We employed trait-based partitioning on genetic variants clusters derived from distance-based hierarchical clustering of their associations with CVD-related traits. Variants were identified from genome-wide association studies for CAD, stroke, HF, PAD, and Afib. Genome-wide significant variants were clustered into 10 partitions, reflecting shared and unique genetic influences across these diseases. Partitioned PRS are evaluated on their associations with common CVDs and subtypes.

Results: This study revealed a structured genetic architecture of CVDs, with 10 partitions capturing unique and overlapping genetic associations underlying biological mechanisms. For instance, a partition shared among CAD, PAD, and stroke is anticipated to predict those diseases and comorbid patients. In contrast, a CAD-specific partition with no association with PAD or stroke is expected to predict CAD only. Similarly, an Afib-specific partition is expected to predict Afib.

Conclusion: This study demonstrates that trait-based hierarchical partitioning identifies shared and unique genetic contributions to common CVDs. The 10 distinct partitions could provide a framework for developing partitioned PRS and enhance genetic risk prediction for individual and overlapping CVDs. These findings could offer valuable insights for improving precision medicine in CVD risk assessment and treatments.

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P07.016.A Impact of rapid Exome sequencing on pediatric patients with cardiomyopathies and acute Heart failure

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Intoduction: Exome Sequencing (ES) is a comprehensive tool for the diagnosis of rare monogenic disorders, but scarce studies address its clinical impact on pediatric cardiomyopathies (PCM) in urgent settings.

Materials and methods: Data was collected retrospectively from medical records of pediatric patients presenting with acute heart failure and cardiomyopathy who underwent singleton rapid ES between 2021 and 2023 at Rambam Medical Center.

Results: A total of nine patients were included. Eight patients (88.8%) presented in the first year of life. Age range 5days-11years, median-6 weeks. Family history was positive in only one case (11.1%). Turnaround Time was 5–14 days (median 9 days). The diagnostic yield was 5/9 (55.5%) confirming primary cardiomyopathy. The majority had dominant disorders (*ACTC1, MYBCP3, TNNI3, NKX2-5*) with two (22.2%) occurring de novo. One patient (11.1%) had a recessive condition (*MYBPC3)*. In Three patients (33.3%) who rapidly deteriorated during admission, a major impact on immediate medical management was imposed: palliative care in one and referral for heart transplantation in two. In most patients, the diagnosis led to avoidance of further metabolic or expert consultations, cardiac magnetic imaging or vitamin treatment. At-risk relatives in three families (33.3%) started cardiac follow-up. In five families accurate recurrence risk was provided.

Conclusions: Our results show a high clinical impact of rapid ES in isolated PCM presenting in urgent settings, including shorter time to diagnosis, high diagnostic yield, improved patient management, and therapeutic approach. In addition to family members at risk segregation and genetic counseling regarding family planning.

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P07.017.B The association between donor-derived cell-free DNA and acute rejection in heart transplant patients

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Background: Lifelong noninvasive rejection monitoring in heart transplant (HT) patients is a therapeutic need still inadequately provided. The historical and still current gold standard for assessment of cardiac acute cellular and antibody-mediated rejection (ACR and AMR) is the endomyocardial biopsy (EMB). Developments in NGS technologies have allowed the concept of liquid biopsy to evolve, with the development of cell-free DNA (cfDNA) analysis in the context of organ transplantation. This study aims to assess the relationship between donor-derived cfDNA (dd-cfDNA) levels, ACR and AMR in a cohort of HT recipients.

Material and methods: dd-cfDNA was extracted from plasma derived from blood EDTA-tubes among the patients subjected to EMB. dd-cfDNA was measured and sequenced on MiSeq (Illumina) platform at >1000X coverage, according to AlloSeq cfDNA protocol (CareDx), which evaluated 202 genomic polymorphisms estimating the percentage of dd-cfDNA, compared to recipient patient, as an index of rejection.

Results: dd-cfDNA assessment was performed in 75 recipients. Starting from 30 days after HT, ACR was detected in 25 (33.3%) patients in whom median dd-cfDNA levels were 0.36% (IQR 0.23 to 0.66%) and AMR was detected in 15 (20.0%) patients, in whom median dd-cfDNA levels were 0.6% (IQR 0.395 to 1.045%). dd-cfDNA levels were higher in recipients with ACR ($p = 0.0169$) and AMR ($p = 0.0002$) *vs*. those without rejection.

Conclusion: In conclusion, starting from 30 days after HT, ACR and AMR were associated with higher dd-cfDNA levels, indicating that this technology can be relevant in detecting HT rejection with minimal invasive tests *vs*. classic EMB.

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P07.019.D Improving genetic testing for heart disease with targeted consideration of population linkage patterns

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Background: Cardiovascular Disease (CVD) is the leading cause of death globally, making it an important target for genetic prediction. Towards this, *LPA* is a promising gene as it influences CVD though Lipoprotein A (Lp(a)), a hereditary blood lipid that is rarely measured in-clinic. Two variants within *LPA* are now used to predict Lp(a) levels, however these have poorer performance in Latin American individuals (LAT). This suggests that these variants are not causal, but rather differentially tagging a causal locus due to differences in linkage disequilibrium across populations. In this case, the putatively causal locus in *LPA* is KIV-2, a repetitive domain rarely included in clinical care or genetic datasets due to inherent challenges in ascertaining repeats. To improve Lp(a) clinical predictions, this motivates us to identify variants tagging KIV-2 well to improve actionability of genetic testing when KIV-2 or Lp(a) data is unavailable.

Material and methods: We identified KIV-2 tagging variants through a generalized linear model correlating *LPA* variants with KIV-2 copy numbers in 1,000 Genomes Project LAT. From this, we designed an improved risk model accounting for a variant tagging KIV-2 plus a Lp(a) polygenic risk score (PRS) built from the UK Biobank.

Results: This method showed a 70% increase in predictive performance for LAT as compared to the standard pruning and thresholding PRS.

Conclusion: Our findings show promise in our ancestry-informed approach, improving Lp(a) genetic testing in the populations most neglected by the current test and providing a framework for more equitable genetic risk prediction overall.

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P07.020.A Unraveling the enigma of DSG2 p.(N266S): a culprit in Arrhythmogenic Cardiomyopathy

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Background: Desmoglein-2 (*DSG2*) truncating variants are major cause of arrhythmogenic cardiomyopathy (ACM), which is an autosomal dominant disease characterized by electrical instability and myocardial fibro-fatty replacement. The clinical significance of rare *DSG2* p.(N266S) missense variant remains an open question (ACMG criteria: PP3, PM2, BP1). The purpose of this study is to explore pathognomonic features of the DSG2-p.(N266S) to support a damaging effect on the gene product (PS3 rule).

Material and methods: An in vitro system based on HL-1 cells and 2 plasmids, carrying either DSG2 Wild-type (Wt) or the p.(N266S) variant, were used to established HL-1-Wt and HL-1-Mut cell lines. Transfection was obtained by 4-D Nucleofector System (Lonza©). For a patient-tailored in vitro system, CD34+ cells were isolated, reprogrammed into induced pluripotent stem cells (iPSCs) and later differentiated into cardiomyocytes (iPSC-CMs).

Results: DSG2 p.(N266S) missense variant was identified in four families exhibiting ACM phenotypes with biventricular involvement, arrhythmias and ECG abnormalities. Transfected HL-1 cells were able to continuously divide, spontaneously contract and expressed both plasmids. Confocal and TEM imaging revealed the insertion of the DSG2 mutated protein into cardiac desmosomes and evidence of gap widening in HL-1 Mut. Calcium flux and the beating phenotype were significantly altered in HL-1 Mut. Desmosomal widening and electrophysiological alterations were confirmed subsequently in iPSC-CMs derived from one proband.

Conclusion: We provided strong evidence to support DSG2 p.(N266S) pathogenicity in ACM through a combination of clinical observations in four independent affected families and further experimental findings in two different in vitro models, HL-1 and iPSC-CMs.

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P07.021.B Familial hypercholesterolaemia: prevalence and discrepancy between genotype and phenotype - results from the population-based Hamburg City Health Study

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Background: Familial hypercholesterolaemia (FH) is one of the most common monogenic diseases, affecting genes involved in low-density lipoprotein (LDL) metabolism. In Germany, there is limited population-based data on the prevalence of genetically confirmed FH (genFH) and genotype-phenotype associations. This study aimed to address this gap using whole-genome sequencing data from the Hamburg City Health Study.

Material and methods: Pathogenic mutations in five FH-associated genes were analysed in 7,373 participants aged 45–74 years. Genotypes were compared to LDL cholesterol (LDL-C) levels, adjusted for lipid-lowering medication. Severe hypercholesterolaemia was defined as LDL-C ≥190 mg/dL.

Results: Heterozygous FH was identified in 23 individuals (0.31%; 1 in 321), all due to *LDLR* mutations. Median treatment-adjusted LDL-C levels were higher in genFH individuals (191 mg/dL [149–210]) than in those without genFH (128 mg/dL [105–153], $p < 0.001$). Severe hypercholesterolaemia was observed in 6.5% of participants, of whom only 2.3% ($n = 11$) had genFH. LDL-C levels were below 130 mg/dL in 9.1% of genFH individuals, below 160 mg/dL in 31.8%, and only 50% had severe hypercholesterolaemia. Screening using LDL-C thresholds of ≥190 mg/dL, ≥160 mg/dL, or ≥130 mg/dL yielded sensitivities of 50.0%, 68.2%, and 90.9%, respectively, with 43, 98, and 175 individuals requiring screening to identify one genFH case.

Conclusion: The prevalence of genFH in this cohort was 0.31%, aligning with global estimates. Significant discrepancies between genFH and LDL-C levels highlight the limitations of genetic FH screening.

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P07.022.C DNA methylation signature in pulmonary arterial hypertension, a potential molecular diagnostic tool

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Introduction: Pulmonary arterial hypertension (PAH) is a rare, life-threatening disorder characterized by increased pulmonary arterial pressure, leading to right heart failure and potential mortality. The incidence ranges from 15 to 50 cases per million annually. Clinical symptoms include fatigue, syncope, chest pain, and edema. While PAH has a multifactorial etiology, genetic and epigenetic contributions play a critical role in its pathogenesis. The diagnostic utility of DNA methylation (DNAm) patterns has emerged as a novel approach to classify and validate genetic variants of uncertain significance (VUS), particularly in genes like **BMPR2, SOX17**, and **EIF2AK4**.

Materials and methods: Using Illumina 950K Methylation Microarray, we analyzed DNAm profiles from 23 patients with pathogenic variants in ***BMPR2*** and 41 controls. Differentially methylated probes (DMPs) were identified, and a support vector machine (SVM) model was employed for classification.

Results: We identified 842 DMPs, with 752 hypomethylated and 90 hypermethylated CpG sites, predominantly outside well-known PAH-associated genomic regions. PCA revealed distinct DNAm profiles between patients and controls. SVM analysis achieved 90% specificity and 100% sensitivity in differentiating patients based on DNAm patterns, with promising results for subclassification among other PAH-related genes.

Discussion: DNAm signatures offer a robust tool for validating VUS in PAH-associated genes, improving diagnostic accuracy. Preliminary data highlight a unique epigenetic signature for ***BMPR2***, distinct from ***SOX17*** and ***EIF2AK4***. Further validation with external cohorts and diverse gene sets is essential to enhance the utility of DNAm profiling as a molecular diagnostic adjunct in PAH.

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P07.023.D The impact of the pro-atherogenic MRAS gene in smooth muscle cells

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A study by Erdmann *et al*. in 2009, revealed a region on 3q22.3, encompassing the *MRAS* gene as a risk factor for CAD. *MRAS* encodes muscle Ras, a GTPase acting as a signal transducer in TNF signalling and other acute phase response pathways. eQTL data suggests *MRAS* risk variants for CAD increase *MRAS* mRNA levels primarily in the arterial tissue in vascular smooth muscle cells (SMCs). However, *MRAS's* exact role in atherogenesis is still elusive. The function of *MRAS* in vascular SMCs was investigated. Human

primary aortic SMCs transfected with *MRAS*-specific siRNA and murine SMCs derived from our *Mras*$^{-/-}$*ApoE*$^{-/-}$ double knockout (dKO) mouse model were subjected to functional assays including proliferation, migration and apoptosis. *MRAS* siRNA knockdown in human SMCs and *Mras* deficient mice SMCs showed increased migration, proliferation and reduced apoptotic activity with and without stimulation of TNF, IL6 and IL1ß. Stimulation with TNF, IL6 and IL1ß enhanced the proliferative effect of *Mras* deficiency, indicating an interplay of these cytokines and MRAS pathways. Bulk RNA sequencing revealed *Mras* KO in murine SMCs led to differential gene expression of numerous genes involved in cGMP/cAMP signalling, suggesting that SMCs upregulate cGMP/cAMP signalling to compensate for the loss of *Mras*. The in vivo atherosclerosis study on plaque characteristic revealed that the impact of *Mras* deficiency was sex-specific as significant differences in plaque size, collagen content and macrophage staining were only observed in male mice. Conclusively, lower MRAS levels in SMCs protects against CAD by enhancing plaque stability and decreasing rupture risk.

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P07.025.B Elucidating the pathomechanisms of Meester-Loeys syndrome: insights from a *Bgn*$^{-/0}$ mouse model

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Background: Pathogenic loss-of-function biglycan (*BGN*) variants are associated with Meester-Loeys syndrome, a thoracic aortic aneurysm and dissection syndrome. To study its pathomechanisms, a *Bgn*$^{-/0}$ mouse model was developed.

Material and methods: Aortic dissection/rupture was induced in *Bgn*$^{-/0}$ mice using a non-hypertensive dose of angiotensin-II (AngII) (400 ng/kg/min). Animals were sacrificed after five days of AngII-infusion, before the occurrence of an aortic dissection/rupture. Descending aortic tissues from AngII- and vehicle-treated *Bgn*$^{-/0}$ and wildtype mice were analysed macroscopically and with histological stainings, transmission electron microscopy, and RNA-sequencing.

Results: Macroscopic and histological analyses revealed red blood cell accumulation within the aortic wall of AngII-treated *Bgn*$^{-/0}$ mice, indicating aortic damage. Verhoeff-Van Gieson and Masson's Trichrome stainings showed no consistent differences in elastin fiber organization or collagen content between groups. Exploratory assessment by transmission electron microscopy revealed variability in collagen fibril diameter, with an overall smaller fibril diameter in *Bgn*$^{-/0}$ mice relative to wildtype mice, regardless of the treatment. This suggests that reduced fibril diameter may reflect a genetic predisposition caused by the biglycan variant, but it does not explain the dissection/rupture phenotype. To further explore the underlying pathomechanisms, bulk and single-cell RNA-sequencing have recently been performed. Principal component analysis highlighted AngII-challenge as the greatest source of variation between samples, suggesting a more heterogeneous response to the environmental challenge compared to the relatively modest effect of the biglycan deficiency. Differential expression analysis is ongoing.

Conclusion: The (preliminary) findings from the *Bgn*$^{-/0}$ mouse model offer valuable insights for further mechanistic studies to unravel drivers of biglycan-related aortopathy.

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P07.026.C Spatial transcriptomics reveal gene expression signature in arterial fibromuscular dysplasia lesions

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Background: Fibromuscular dysplasia (FMD) is a group of arterial lesions (stenosis and aneurysms), which present in hypertension, stroke and spontaneous coronary artery dissection (SCAD), an under-studied form of myocardial infarction. With ~50% of SCAD patients present extra-coronary FMD, in both conditions cardiovascular risk factors and high proportion of women are found. Here we aimed to explore gene expression signatures in human arteries to delineate FMD specific arterial remodeling.

Material and methods: We selected 6 FMD and 2 non-FMD formalin-fixed paraffin-embedded arterial samples for 10X Visium spatial transcriptomic sequencing (Illumina). FASTQ files were processed using Space Ranger (v3.0.0), differential expression using Seurat (v5.0.3) in R (v4.3.3), and enrichment analyses using clusterProfiler (v4.10.1). Cell types were inferred using public human artery single-cell RNAseq datasets.

Results: After quality control, we analyzed six samples (4 FMD, 2 non-FMD) with an average of 2,787 spots per sample and mapping rates above 97%. Clustering identified 10 groups, with M1-M5 in the media and A1-A5 in the adventitia, with intima layer virtually uncaptured. M1, M2, M3 and M5 were enriched in smooth muscle cells (SMCs) expressing *MYH11* and *TAGLN*. Collagen-enriched M3 was predominant in FMD lesions (7.5% vs. 0.3% in non-FMD). M4, unique to FMD, displayed a macrophage-like signature (*CD68*). Pathway analyses revealed upregulation of genes linked to extracellular matrix biology in FMD lesions while contractile fiber genes were downregulated.

Conclusion: Our findings suggest increased fibrosis and impaired SMC function in FMD. Ongoing histological validation will further elucidate SMCs remodeling mechanisms, in comparison to atherosclerosis.

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P07.027.D In vivo interrogation of the contribution of *Igfbp2* upregulation to thoracic aortic aneurysm development

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Background: Thoracic aortic aneurysm (TAA), the abnormal widening of the thoracic aorta, can occur in isolation or as part of a connective tissue disorder like Marfan syndrome (MFS). Insulin-growth factor binding protein 2 (Igfbp2) regulates cell proliferation, growth, and extracellular matrix remodeling. Our lab identified significant upregulation of *Igfbp2* in the aortic wall of three independent TAA mouse models (including *Fbn1*$^{C1041G/+}$ (MFS)), with an 8- to 30-fold increase prior to aneurysm development, suggesting that it might drive TAA establishment.

Material and methods: We explored the role of upregulated *Igfbp2* on TAA development and progression by crossbreeding *Igfbp2*$^{+/-}$ and *Fbn1*$^{C1041G/+}$ mice. This breeding yielded pups belonging to four genotype groups (N = 14 per group): *Fbn1*$^{+/+}$/*Igfbp2*$^{+/-}$, *Fbn1*$^{C1041G/+}$/*Igfbp2*$^{+/+}$, *Fbn1*$^{C1041G/+}$/*Igfbp2*$^{+/-}$, and *Fbn1*$^{+/+}$/*Igfbp2*$^{+/+}$. Aortic root and ascending aorta diameters of male mice were determined every 4 weeks from 4 to 28 weeks of age via transthoracic echocardiography utilizing a Vevo-2100 system with MS550D transducer (VisualSonics). Aortic root and ascending aorta data were analyzed separately using likelihood ratio tests and chi-squared statistics. Post-hoc analysis involved a Tukey's HSD test.

Results: Our analysis confirmed exaggerated aortic growth both at the level of the aortic root and the ascending aorta in male *Fbn1*$^{C1014G/+}$ versus wildtypes. In contrast, haplo-insufficiency for *Igfbp2* alone showed no measurable effect on aortic diameter, nor did it have an impact in combination with an *Fbn1*$^{C1041G/+}$ background.

Conclusion: Haplo-insufficiency for *Igfbp2* in *Fbn1*$^{C1041G/+}$ mice has no significant impact on TAA development and progression, suggesting that it may not be sufficient to mitigate aneurysm progression.

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P07.028.A Development and characterization of an HHT-iPSCs model for drug testing

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Background: The latest gene editing technologies and the use of iPSCs is leading to the development of novel cell-based disease models that allow to reproduce and characterize the phenotypic alterations implied in the pathogenesis of specific disorders. Hereditary Hemorrhagic Telangiectasia (HHT) is a rare genetic vascular disease caused by mutations in *ENG*, *ACVRL1*, *SMAD4*, or *GDF2*, leading to an impairment of the TGFβ/BMPs pathway. Here we present an iPSCs-based cell line carrying a homozygous truncating variant in *ENG*, obtained by CRISPR-Cas9 technology.

Material and methods: CRISPR-Cas9 was performed on the iPSCs LUMC0072 line. The quality of the gene editing was attested by restriction enzyme digestion, Sanger sequencing, Short Tandem Repeats, karyotyping, pluripotency and multi-lineage assays. The lines were mycoplasma free. Angiogenesis studies were performed on both parental and mutated lines, which were further differentiated in endothelial cells and blood vessels organoids (BVOs). Proliferation assays and migration under laminar flow were conducted.

Results: The quality assessment of LUMC0072-F5 *ENG*$^{mut/mut}$ line, carrying the *ENG* c.1A>G truncating variant, was successful. The in vitro HHT model showed defective angiogenic features, including flow-induced cell migration and response to VEGFR2 signalling, comparable to the already published data on Eng-KO mice retina.

Conclusion: The development of a reliable and highly characterized in vitro HHT model represents an important tool for deepening the cellular and molecular processes modulating angiogenesis, leading to the pathological phenotype. Furthermore, this may provide a valuable support for drug testing.

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P07.029.B Current WES-trio analysis does not reveal genetic cause of idiopathic neonatal arterial ischemic stroke: implications for future research

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Background: Neonatal arterial ischemic stroke (NAIS) is a cerebrovascular condition characterized by a localized disruption of cerebral blood flow due to artery obstruction, which can yield prolonged neurological consequences. Despite research aimed at elucidating the genetic basis of stroke in pediatric and adult populations, the precise contribution of genetic factors in the pathogenesis of NAIS remains unknown.

Material and methods: Genetic analysis of newborns with NAIS was performed using WES-trio. A personalized workflow was applied for filtering, analyzing, and interpreting genetic variants in de novo dominant and autosomal recessive models. Functional studies included plasmid recombination, cell culture, western blot, and confocal microscopy.

Results: In the WES-trio analysis of 23 newborns with idiopathic NAIS we identified 28 unique de novo variants in 28 genes. No variant was diagnostic quality. The *PIK3CD* variant c.1292A>G/p.[Gln431Arg], from a patient with perforant stroke, was selected as the strongest candidate possibly linked to NAIS. Functional studies overexpressing both wild type and p.Gln431Arg PIK3CD proteins suggested this variant might disrupt protein function. However, no significant PI3K/Akt signaling pathway changes were detected. WES-trio analysis under the autosomal recessive model did not allow the identification of candidate genes.

Conclusion: The study did not provide conclusive evidence for a genetic cause of idiopathic NAIS. However, it suggested that genetic variations, particularly in *PIK3CD*, might still play a role in perforant stroke. We cannot completely rule out a genetic basis, as relevant variants may lie outside protein-coding regions, requiring alternative genomic approaches, including whole genome sequencing (short/long reads) and RNA-seq.

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P07.030.C Analysis of the *MYBPC3* spectrum in hypertrophic cardiomyopathy with a focus on splicing variants

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Background: a genetic etiology is present in ~30% of patients with hypertrophic cardiomyopathy (HCM), *MYBPC3* being the most frequently involved gene. We analyzed a cohort of molecularly characterized HCM patients, focusing on the spectrum and impact of *MYBPC3* variants.

Material and methods: between 2018 and 2024, 520 HCM patients underwent next-generation sequencing (NGS) of a panel of HCM-related genes. *MYBPC3* genotype-phenotype correlations were assessed. Selected *MYBPC3* splicing variants were studied using cDNA sequencing.

Results: Genetic analysis identified variants in 286 patients (55.0%): 133 (46.50%) carried pathogenic (C5)/likely pathogenic (C4) variants, and 153 (53.50%) had variants of uncertain significance (VUS). A total of 109 *MYBPC3* variants were detected in 102/520 (19.6%) patients, 83 of whom presented with classic HCM phenotype and 19 with an atypical phenotype. Of the 109 *MYBPC3* variants, 62 (56.9%) were causative (C4/C5), and 47 (43.1%) were VUS. In total, 68/109 (62.39%) *MYBPC3* variants were truncating, 41 (37.61%) were missense without predicted splicing impact. Furthermore, 24 intronic and 34 exonic *MYBPC3* variants (58/109, 53.2%) were predicted to affect splicing. Although statistical significance was not reached, truncating *MYBPC3* variants were often associated with a more severe phenotype and earlier onset than other variants. Cloning and sequencing from mRNA obtained from peripheral blood mononuclear cells determined defective splicing for 3 variants, previously classified as C3/C4, allowing variant reclassification.

Conclusion: NGS is a powerful tool for molecular diagnosis in HCM, but VUS remain a challenge. Functional studies play a critical role in reclassifying VUS, improving the clinical utility of genetic testing.

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P07.031.D Panel-negative cases diagnosed with WGS in patients with cardiomyopathy and arrhythmia

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Background: Current cardiological guidelines emphasize the use of targeted gene panels in diagnostics focused on known disease-associated genes, potentially overlooking diagnostically relevant variants in other genomic regions. In contrast, whole-genome sequencing (WGS) offers a comprehensive approach, enabling targeted analysis of genes linked to a patient's phenotype and the broad detection of genetic variants.

Methods: We employed WGS to investigate a cohort of 21 patients with suspected monogenic cardiovascular disease (CVD), in which previous gene panels failed to identify (likely) pathogenic variants between 01/2015 and 03/2022. The analysis included a comprehensive search for variants across the entire genome, focusing on genes relevant to the clinical phenotypes. The patients were classified according to the following phenotypes: cardiomyopathy (n = 18) and arrhythmia (n = 3).

Results: WGS identified variants in 71.4% (15/21) of cases. 53% (8/15) were classified as (likely) pathogenic, implicating them as causative of the patients' phenotype. The remaining 47% (7/15) were categorized as variants of uncertain significance. In 66.6% (10/15) of cases, the affected gene was not included in the panel analysis. In 20% (3/15) of cases, the variant was also reported but considered causative for the phenotype. In a single instance, the affected gene was included in the panel but the variant was not reported. In the remaining case, the unreported variant was located in an intronic region.

Conclusion: This study highlights the enhanced diagnostic yield of WGS compared to conventional gene panels for patients with suspected monogenic CVD. Our results support the routine use of genome wide analysis.

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P07.032.A Mutations in angiogenesis-related genes, revealed by whole exome sequencing in patients with congenital cutaneous arterial, capillary and lymphatic malformations

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Background: Cutaneous vascular malformations (CVMs) are congenital anomalies resulting from errors in vascular development, often presenting as capillary, venous, lymphatic, or mixed malformations. While the genetic basis of CVMs is increasingly recognized, many causative mutations remain unidentified. This study aimed to uncover novel genetic mutations associated with CVMs using advanced genomic techniques in a cohort of affected individuals.

Material and methods: Whole-exome sequencing (WES) was performed on 9 patients diagnosed with CVMs.

Results: In 7 out of 10 patients (70%) the following genetic variants were detected: pathogenic/likely pathogenic (*FLT4* [c.3341C>T], *VEGFC* [c.148-3_148-2delCA]; *EIF2AK4* [c.4065+1G>T];

JAG1 [c.3003_3006dupTTGC]) and VOUS (*NOTCH1* [c.7583A>T], *RASA1* [c.1583A>G]; *FLT4* [c.3002C>T]). We identified 3 novel missense mutations in the following genes: *NOTCH1* - a critical regulator of arterial differentiation and blood vessel (BV) sprouting; *RASA1* - gene functioning downstream of the Ephrin B4 receptor to control BV development; and *FLT4* - gene regulating the development and maintenance of the lymphatic system. EIF2AK4 mutation, classically found in pulmonary capillary hemangiomatosis and hereditary pulmonary arterial hypertension was found in a patient with cutaneous hemangiomatosis. The segregation analysis was performed in the healthy parents and none of them was a carrier of the corresponding variants, confirming they are de novo.

Conclusion: This study highlights novel genetic mutations associated with CVMs, advancing our understanding of their molecular etiology. These findings underscore the potential for genomic approaches in enhancing diagnostic accuracy and informing therapeutic development. Further research is needed to investigate the functional consequences of these mutations.

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P07.033.B A Retrospective analysis of *MYBPC3* variants - optimized bioinformatics pipeline reveals pathogenic intronic variants in Former Genotype-Negative HCM cases

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Background: Genetic variants in the *MYBPC3* gene are among the most common genetic causes of hypertrophic cardiomyopathy (HCM). Recent studies have identified causal intronic variants, introducing cryptic splice-sites located in outside the standard regions of interest of genetic testing. By default, intronic regions outside the canonical splice site (± 8 bp) are not analyzed in the daily practice of our diagnostic laboratory. The aim of this work was to conduct a retrospective analysis and optimize the workflow used in routine cardiogenic testing to include the study of important intronic regions.

Material and methods: A retrospective study was conducted on a cohort of 658 HCM probands, through the development of a bioinformatics pipeline that allowed us to search for five known intronic *MYBPC3* relevant variants (c.1224-19G>A, c.1224-52G>A, c.1224-80G>A, c.1224-21A>G, and c.906-36G>A).

Results: This analysis allowed the detection of 4 cases with splicing variants (0.6%) that were previously missed using the conventional approach. All cases were otherwise genotype-negative.

Conclusion: These results emphasize the importance for laboratories to regularly update their bioinformatics pipelines, incorporating the study of well covered intronic regions, in genes like *MYBPC3*. This strategy can increase the detection of pathogenic/likely pathogenic variants, improving HCM diagnostic yield, without increasing costs or turnaround time for genetic testing.

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P07.034.C Copy number analysis in Czech patients with the inherited cardiac diseases by next generation sequencing

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Background: Copy Number Variants (CNVs), including exon deletions and duplications, are significant contributors to the pathogenesis of various genetic diseases. These structural alterations can disrupt gene function, dosage, or regulation, leading to a wide spectrum of disorders, including cardiomyopathies and arrhythmogenic syndromes. Recent advances in genetic testing technologies, particularly next-generation sequencing (NGS), have enhanced the detection of these variants, improving diagnostic accuracy and risk assessment.

Material and methods: Our study involved targeted sequencing of genes associated with inherited cardiac diseases, along with bioinformatics analysis for detecting small-scale gene variants and CNVs.

Results: We present genetic findings in Czech patients with cardiomyopathies and arrhythmogenic syndromes. Pathogenic or likely pathogenic variants were identified in 104 unrelated patients (26.3%). CNVs - specifically whole-exon deletions within genes - were detected in 4 patients in the genes *RYR2*, *JUP*, *NEXN*, and *SNTA1*. Notably, exon deletions in *JUP* and *SNTA1* have not been previously described. Deletions involving the entire *NEXN* gene have been reported in the literature; however, we detected a novel deletion involving exons 2–5 in a homozygous state in a patient with heart failure in infancy. This finding broadens the spectrum of patients with recessive inheritance in the *NEXN* gene.

Conclusion: The identification of novel CNVs in genes such as *JUP*, *SNTA1*, and *NEXN* expands our understanding of the genetic basis of inherited cardiac diseases. These results underscore the importance of comprehensive genetic testing, including CNV analysis, in improving diagnostic precision and patient care.

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P07.036.A Mainstream genomic testing is acceptable to cardiologists: the development and evaluation of a Genetic Counsellor led program in an Australian metropolitan hospital

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Background: Genetic Services have limited capacity to meet the growing demand for diagnostic genomic medicine. Mainstream

genomic testing is an alternative model of care, however, specialty-specific training in genetics is critical if precision medicine is to be embraced.

Material and methods: A mainstream genomic testing program for patients at risk of inherited cardiomyopathy, arrhythmia and familial hypercholesterolemia was developed. A Genetic Counsellor was embedded in select cardiology clinics for 9 months to train and support Cardiologists to provide genomic testing to patients. Cardiologists' experiences were assessed through completion of quantitative evaluation surveys pre- and post- the Genetic Counsellor embedment. The program, resources and evaluation survey were developed through an iterative process, informed by established mainstream programs and adapted from validated implementation measures.

Results: Twenty-three Cardiologists completed at least one evaluation survey. Cardiologists' familiarity with testing processes doubled ($p < 0.001$), they reported increased confidence with the practical requirements of testing ($p < 0.001$), their ability to interpret test reports ($p = 0.006$), and to discuss testing with patients ($p < 0.001$). By the second survey, all Cardiologists reported the use of mainstream testing was acceptable. The success of the program was further evidenced by an 11% pathogenic variant detection rate and Cardiologists' continued use of mainstream testing 8 months after the embedment period ended.

Conclusion: In under 12 months, the program successfully supported the adoption and ongoing use of genomic testing in a cardiology unit. This demonstrates how Genetic Counsellors can partner with specialty clinics to provide support, education and training, and establish a sustainable mainstream genomics program.

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P07.037.B Spontaneous coronary artery dissection and a family history of aortic dissection: a genetic association study

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Background: Spontaneous coronary artery dissection (SCAD) is a cause of acute coronary syndrome, primarily affecting relatively young women (median age:51years) without typical cardiovascular risk factors. SCAD has a genetic component, with recent genome-wide association studies identifying multiple risk loci. Thoracic aortic dissection (type A), another vascular disorder, shares some genetic overlap with SCAD, suggesting potential common genetic predispositions.

Material and methods: We performed genetic screening or whole genome sequencing (WGS) of 17 SCAD cases (94% women) who had a family history of aortic dissection (AD) in a first- or second-degree relative (89% men). We assessed rare variants in candidate genes and genome-wide using the American College of Medical Genetics and Genomics criteria. Polygenic risk scores (PRS) were calculated to assess the cumulative genetic risk for SCAD, fibromuscular dysplasia, AD, and abdominal aortic aneurysm (AAA) in SCAD cases, relatives with AD, and controls.

Results: WGS identified pathogenic/likely pathogenic variants in *SMAD3*, *CBS* and *COL3A1* genes associated with aortopathies in three SCAD cases. Four variants of uncertain significance were found in candidate genes. PRS for SCAD were significantly associated with increased odds of SCAD in probands compared to controls (OR = 1.79, 95%CI = 1.08–2.99, p = 0.024).

Conclusion: Our study supports the complex genetics underlying SCAD, implicating rare pathogenic variants and polygenic risk. We identified pathogenic variants in SCAD cases with a family history of AD, highlighting potential genetic links between these vascular disorders. Findings underscore the importance of genetic screening in families of patients with SCAD and family history of AD to identify at risk individuals.

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P07.038.C Long-Read RNA sequencing in cardiomyopathies : a new approach for genetic diagnostic with strong potential ?

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Background: Dilated cardiomyopathies (DCM) are inherited diseases, for which genetic diagnostic conditions patient follow-up and family care (major gene *TTN*). However, current DNA Short Read (SR-DNA-Seq) based sequencing diagnostic yield is only 15–25% suggesting methodological limitations. This lack might be mitigated by RNA Long-Read (LR-RNA-Seq) which detects elusive DNA rearrangements and cryptic aberrant splicing causative mutations. We aim to evaluate LR-RNA-Seq for detection of Single Nucleotide Variants (SNVs) in coding exons and short/complex DNA Structural Variants (SV).

Material and methods: Cardiac RNA from 26 DCM cases were sequenced on a PromethION24 (ONT). SNVs and SV were called using a LR dedicated bioinformatic tool (Clair3). True variants calling thresholds were inferred from replication using DNA Sanger.

Results: Samples were covered with 11.6M reads (mean size:1094pb). Among 33 LR-RNA-seq variants, 11 were confirmed in DNA, allowing to define key parameters witnessing for high prior probability variants. All 3 causal SNV already diagnosed were detected. We detected 2 new causative truncating variants in the TTN gene in 2 other patients. Finally, we identified rare monoallelic variants overlapping with TTN locus, suggesting hemizygosity confirmed by Genomic DNA qPCR. Overall, the diagnostic yield of LR-RNA seq was 23% (6/26), at the upper limit of the reported yields for DCM.

Conclusion: LR-RNA-seq appears to be an efficient method to detect coding variants in expressed genes, as well as SV. Ongoing analysis of splicing and NMD-related variants in our LR-RNA-seq data might further increase diagnostic yield, potentially representing a key strategy to complement SR-DNA-Seq in genetic diagnostic.

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P07.039.D RNA-sequencing unveils novel *FLT4* splice site variants in isolated CHD

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Background: Congenital heart disease (CHD) affects more than 8/1000 live-born babies and may occur isolated or as a syndromic constellation. The pathophysiology is complex, involving genetic and environmental contributions, with unknown etiology in most cases. Heterozygous loss-of-function (LOF) variants in *FLT4* have recently been linked to Tetralogy of Fallot (TOF). We report two families with LOF variants in *FLT4*, contributing to the molecular etiology of isolated CHD.

Material and methods: In 318 probands with isolated CHD and normal CNV analysis, we performed trio exome sequencing on genomic-DNA using a virtual CHD panel of 471 genes. *FLT4* (NM_182925.5) variants were classified using ACMG guidelines. We applied RNA-sequencing on lymphocytes to evaluate to evaluate splicing-effects.

Results: We identified novel PVs in *FLT4* in two families with non-syndromic CHD. In family one, two siblings diagnosed with TOF and MAPCAs harbored a heterozygous c.985+1G>A *FLT4* variant, inherited from their asymptomatic father. In family two, the proband presented with bicuspid aortic valve and aortic coarctation and harbored a heterozygous c.1657+6T>C variant in *FLT4*. The variant was inherited from her asymptomatic mother, but occurred de novo in the maternal grandmother diagnosed with TOF. For both variants RNA-sequencing revealed skipping of exon 7 and 12 in the c.985+1G>A and c.1657+6T>C variant respectively, resulting in a premature termination codon, reclassifying both variants as pathogenic. Previous targeted RT-PCR analysis for the c.1657+6T>C variant revealed normally.

Conclusion: Our report expands the clinical spectrum of heterozygous *FLT4* splice-site variants with reduced penetrance. RNA-sequencing is a powerful tool to uncover missing heritability in isolated CHD.

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P07.040.A Selenoprotein deficiency disorder causes a lethal perinatal cardiomyopathy

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Background: The SECIS binding protein 2 (*SECISBP2*) gene is required for the synthesis of selenoproteins, potent antioxidants that regulate inflammatory signalling pathways and modulate levels of reactive oxygen species. Rare biallelic *SECISBP2* variants have been associated with pleiotropic phenotypes, including abnormal thyroid hormone metabolism, neurodevelopmental disorders and aortic aneurysm. Here, we report a consanguineous South Asian family with a history of three perinatal deaths due to progressive cardiomyopathy and arrhythmias with a rare homozygous splice site variant in the *SECISBP2* gene.

Material and methods: DNA from the parents and four offspring underwent whole-genome sequencing as part of the Elusive Hearts study of families with likely monogenic cardiac diseases. Genome-wide analysis on GATK joint-called VCFs was performed using seqr, and biallelic autosomal variants prioritised. RNA sequencing was performed on parental blood samples and stored fibroblasts from two cardiac-affected offspring.

Results: The rare NM_024077.5(*SECISBP2*):c.1303-2A>G variant (gnomADv4 AC = 2) was heterozygous in parental samples, and homozygous in three deceased offspring. A fourth baby without cardiac disease did not carry the *SECISBP2* variant. RNAseq confirmed this is a loss of function variant, with an in-frame event due to use of a cryptic splice site only seen in ~1% of transcripts. Keshan disease is a progressive cardiomyopathy resulting from nutritional selenium deficiency, and provides a precedent for associating disorders of selenoprotein biosynthesis with cardiac disease.

Conclusion: We report the first instance of perinatal lethal cardiomyopathy due to biallelic variants in *SECISBP2*, causing a more severe phenotype than previously reported cases linked to this gene.

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P07.042.C Whole exome sequencing in pediatric cardiomyopathies

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Background: Pediatric cardiomyopathies are a group of rare diseases that include hypertrophic, dilated, restrictive, arrhythmogenic cardiomyopathies and left ventricular non-dilated cardiomyopathies. While their causes are well understood in adults, genetic causes in children are less known for both isolated and syndromic forms.

Material and ethods: We conducted a retrospective study (2018–2024), analyzing sequencing data from 59 patients aged <18 years old who underwent genetic testing for cardiomyopathy as index cases at the University Hospitals of Amiens and Lille. The main objective of this work was to explore the spectrum of genes involved in pediatric cardiomyopathies using solo Whole Exome Sequencing (WES).

Results: The detection rate of relevant variants identified through solo WES was 64.4% (38 out of 59 patients). Among these, pathogenic/likely pathogenic (P/LP) variants were identified in genes that are rarely described but known to cause isolated cardiomyopathies or to be involved in syndromic diseases. Moreover, two patients with severe disease had two P/LP variations in two different genes.

Conclusion: When compared with panels made on evidence-based assessment of genes, the results of our study show variations in genes that are missing from these panels. This confirms the need for extended genetic analysis in children with cardiomyopathy, even if it appears to be isolated, especially in cases of early presentation, to refine their genotypic spectrum. Furthermore, double mutations in sarcomeric genes are more severe than single mutation and we should systematically look for a second mutation in cases of paediatric presentation.

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P07.043.D Beyond dilated cardiomyopathy: expanding the clinical spectrum of TTN truncating variants

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Background: TTN truncating variants (tvTTN) are known to cause dilated cardiomyopathy (DCM), but their role in other cardiomyopathies remains unclear. It is not uncommon to observe non-DCM phenotypes in patients with tvTTN, and accurate classification of these variants is crucial for diagnosis, especially regarding the PVS1 criterion. This study aims to expand the clinical spectrum of tvTTN non-DCM phenotypes.

Methods: We reviewed cases with non-DCM tvTTN from January 2017 to January 2025. Patients were studied using NGS-based cardiomyopathy panels or whole exome sequencing. Assuming the N2A transcript (NM_133378.4), all variants were matched to its exonic location, gene region, and PSI scores.

Results: We identified 11 cases, including eight with suspected hypertrophic cardiomyopathy (HCM), one with myofibrosis, one with left-ventricle non-compaction, and one neonatal death. Of the eight HCM cases, four variants were in the A-band, two in the M-band, and two in the I-band, with all but one [c.24248C>G p.(Ser8083*)] having a PSI of 100. The neonatal death case had an intronic variant (c.296-2A>T) in the Z-disc region. The myofibrosis case had a variant in the I-band with a PSI of 6.

Conclusion: Our findings confirm the wide spectrum of cardiomyopathy-related phenotypes that have been described in patients with tvTTN. These results alone do not establish genotype-phenotype associations, but provide further clues for building a more complete picture of the consequences of truncating variants in this gene, which have been particularly difficult to predict.

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P07.044.A Clinical laboratory correlation improves the reporting strategy of variants of uncertain significance in hereditary cardiomyopathies and heart rhythm disorders

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Background: besides the ClinGen's efforts to standardize the ACMG/AMP criteria and European initiatives aimed at monitoring quality standards, molecular diagnostics of hereditary cardiomyopathies and heart rhythm disorders (HCHRDs) remains strongly influenced by the local strategies developed to overcome the variables in which genetic testing is requested. This is a monocentric study on the clinical and molecular findings of 363 pedigrees with various HCHRDs.

Material and methods: ACMG/AMP criteria were adapted according to the ClinGen's material and internal specifications, and the significance of selected variants was supported by RNA studies. Genotype-phenotype data were scrutinized by considering the concurrence of multiple variants in the same individual, disease-gene associations and the effect of selected clinical criteria.

Results: one or more (likely) pathogenic variants were found in 80 pedigrees (22.0%), while 96 (26.4%) displayed one or more variants of uncertain significance (VUS) only. The 132 identified VUS were subclassified according to the Bayesian score in three categories presenting distinguishable patterns of selected criteria. VUS_high showed profiles of key molecular criteria and resembled deleterious variants according to the combinations of assigned criteria, while the VUS_low category displayed a high chance of conflicting combinations of criteria and unsupported disease-gene associations. Reclassification to likely pathogenic by the application of applicable clinical criteria (PVS1_Strength, PP1 and PP4) was accessible to VUS_high and a few VUS_mid only.

Conclusion: this work represents a preliminary support to the clinical utility of VUS subclassification in HCHRDs.

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P07.045.B Molecular autopsy in sudden cardiac death: conclusion of "cold-cases"

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Background: Sudden cardiac death (SCD) occurs within an hour of symptom onset in apparently healthy individuals (1:100,000 in <35 years, annually), profoundly impacting families. Structural heart diseases and channelopathies, often genetically driven, contribute significantly. When medical history is unremarkable and autopsy findings are minimal, genetic testing aids diagnosis and risk assessment for the family care, though high-quality DNA from autopsy samples poses great challenges.

Material and methods: 114 SCD patients who underwent autopsy were subjected to Whole Exome Sequencing (WES). DNA was extracted from blood (n = 61) and from formalin-fixed paraffin-embedded (FFPE) tissue (n = 53) (average FFPE time = 8.8 years). Variant prioritization and classification followed American College of Medical Genetics and Genomics guidelines.

Results: FFPE tissue was a suitable starting material in 53% of samples (28/53). Interestingly, DNA quality showed no correlation with the year of tissue inclusion. Genetic variants in CMP-associated genes were detected in 45% of cases (40/89), with only 14 (16%) classified as pathogenic/likely pathogenic (P/LP). Notably, 50% of P/LP variants (n = 7) were found in the FFPE subcohort, resulting in a 25% positivity rate. Family co-segregation analysis identified two families where the SCD victim carried two distinct variants, each inherited from a parent.

Conclusion: Determining the genetic cause of SCD enables a more precise diagnosis, provides essential closure for families, and aids in assessing SCD risk in surviving relatives. Effective DNA isolation from FFPE SCD samples facilitates high-quality genetic analysis, playing a crucial role in resolving "cold cases" through molecular autopsy.

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P07.046.C The role of compound heterozygous variants in cardiovascular disease: insights from a cohort of 345 CVD families

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Background: Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality, with significant genetic contributions. While dominant inheritance models have been extensively studied, the role of autosomal recessive variants in CVD remains underexplored. This study analyzed genomic data from CVD-affected patients and their families to evaluate recessive compound heterozygous variants.

Material and methods: The trio genome sequencing data of 345 CVD-affected patients and their parents were retrospectively analyzed. A Recessive Potential Compound Heterozygous annotation framework identified candidate variants based on rarity (allele frequency≤0.0015 in 1000 Genomes and gnomAD) and quality. Inheritance configurations were determined using custom rules incorporating phasing and parental genotype matching. Fisher's exact test evaluated gene-level statistical significance to identify disproportionately impacted genes.

Results: Our analysis identified that compound heterozygous variants in TTN (FDR:0.0001) and OBSCN (FDR:0.0315) are significantly enriched in the CVD cohort. TTN, encoding titin, is essential for cardiac muscle integrity, and mutations in TTN are established causes of Dilated Cardiomyopathy (DCM), Hypertrophic Cardiomyopathy (HCM), and Left Ventricular Non-Compaction (LVNC)[1]. Similarly, OBSCN, encoding obscurin, plays a crucial role in muscle organization, with variants linked to HCM, DCM, and LVNC[2]. Our findings are consistent with previous studies and further validate their pathogenic relevance in CVD.

Conclusion: This study profiles the configuration of compound heterozygous variants in a CVD cohort. Validated by the identification of TTN and OBSCN as significant contributors, our results underscore the impact of compound heterozygosity in CVD. These findings provide insights into CVD genetics and offer potential implications for diagnostics and therapeutics.

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P07.047.D *TTN*:c.12478del in proximal I-band of titin represents a common molecular cause of dilated cardiomyopathy in Slovenian patients

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Background: Titin truncating variants (*TTN*tv-s) are the most common cause of dilated cardiomyopathy (DCM). Little is known about *TTN*-pathy in Slovenian patients with DCM. We aimed to investigate the relevance of *TTN*tv-s in Slovenian probands with DCM.

Material and methods: An internal database of exome sequencing results was used to identify probands with DCM. Their data were screened for (likely) pathogenic (LP/P) variants. Whole genome sequencing was used to investigate the founder effect of the most commonly identified variant. Patients' medical and family histories were collected retrospectively.

Results: 211 probands with DCM were identified, of whom 60 (27.1%) had a LP/P variant. Thirty-three *TTN*tv-s were classified as LP/P, 13 in I-band (19 probands) and 20 in A-band (25 probands). Thus, LP/P *TTN*tv-s explained 73.3% of the Slovenian molecular pathology of DCM. The most frequently identified variant was *TTN*:c.12478del, identified in 7 individuals (15.9%), affecting the I-band (cardiac-specific N2Bus region), suggesting its founder origin. These individuals had severely reduced left ventricular ejection fraction, three of whom also had tachyarrhythmia.

Conclusion: We observed a high yield of LP/P *TTN*tv-s in Slovenian probands with DCM. We found that a significant contribution to *TTN*-related DCM in the Slovenian population is due to a recurrent I-band variant. This highlights the importance of understanding population-specific genetic variability in the diagnosis of DCM.

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P07.048.A Biallelic GNPTAB variants are a rare cause of arrhythmogenic cardiomyopathy and sudden cardiac death

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Background: Pathogenic variants in the N-acetylglucosamine-1-phosphate transferase subunits alpha and beta (GNPTAB) are definitively associated with autosomal recessive mucolipidosis type II/III; a rare lysosomal storage disorder including skeletal dysplasia at birth (type II) or childhood (III). Cardiac features have been described, with shortened life expectancy due to cardiorespiratory failure. We report siblings with sudden cardiac death (SCD), arrhythmogenic cardiomyopathy (ACM) and skeletal dysplasia; with two heterozygous variants in GNPTAB.

Material and methods: DNA from the affected siblings underwent whole-genome sequencing as part of the Elusive Hearts study. Genome-wide analysis on GATK joint-called VCFs was performed for both siblings, with monoallelic/biallelic autosomal variants fitting known OMIM-inheritance associations prioritised in seqr.

Results: The NM_024312.5(GNPTAB):c.3503_3504del p.(Leu1168Glnfs*5) and c.1400A>G p.(Asp467Gly) variants were heterozygous in both affected siblings; and strongly inferred to be in trans based on variant co-occurrence data from gnomAD v2. The c.3503_3504del variant is pathogenic and a known Quebec founder; while c.1400A>G is present 6 times in gnomADv4 and classified as uncertain significance. While cardiorespiratory failure is reported, only two reports of dilated cardiomyopathy exist. Our sib pair includes a male with epiphyseal dysplasia in childhood and SCD aged 32years with cardiomegaly, abnormally shaped vacuolated myocytes, extensive fibrosis and fat infiltration of the RV. His sister had congenital hip dysplasia, ACM in her 30's, impaired RV, ventricular tachycardia on loop recorder.

Conclusion: We report an atypical presentation of GNPTAB-mucolipidosis type III, with ACM as the primary cardiac presentation, and mild skeletal dysplasia. GNPTAB should be considered in sudden cardiac death gene panels.

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P07.049.B 'Impact of novel RAMBAs on cardiac development'

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Background: Congenital Heart Diseases (CHDs) are the most common class of newborn defects and despite their high prevalence, the underlying mechanisms and genes involved are not yet clear. Retinoic acid (RA) is the most active metabolite of Vitamin A and has been identified as an important signalling molecule during vertebrate cardiac development. RA binds to nuclear receptors to regulate transcription. Bioavailability of RA is regulated by the balance between two enzyme classes which synthesise (Retinoic acid dehydrogenases-RALDHs) and metabolise (cytochrome p450 subfamily 26- CYP26s) RA. Both the excess and deficiency of RA produce similar heart defects in the embryo.

Material and methods: Two novel Retinoic acid metabolism blocking agents (RAMBAs), C2 and C17, and Talarozole (R115866) which inhibit CYP26 function were used to induce RA excess at 6hpf until 24 and 72hpf. RA signalling inhibition was induced with the similar treatment BMS195614 (Retinoic Acid Receptor α Antagonist (RAA)). Cardiac development was analysed by molecular makers for cardiomyocyte specification, with Fisher's exact Test used to determine statistical significance.

Results: All RAMBA-treated embryos show defective cardiac chamber formation and looping, indicating in vivo action of the novel RAMBAs and a role for CYP26 enzymes in zebrafish cardiac development. C17 displays higher potency in affecting cardiac specification than C2. Similar defects were seen in RAA-treated embryos, plus cardiac domain posteriorisation.

Conclusion: This research has demonstrated for the first time the in vivo effect on cardiac development of two novel RAMBAs, shedding light on RA signalling and metabolism during cardiac development.

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M.C. Altomonte: None. **A.M. Alex:** None. **D. Durmus:** None. **C. Roberts:** None.

P07.050.C Mutation burden for familial hypercholesterolemia in Bulgarian individuals

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Background: Familial hypercholesterolemia (FH) is a group of inherited genetic defects that result in severely elevated serum cholesterol concentrations due to delayed removal of low-density lipoprotein (LDL) from the blood in hepatocytes. The cause is autosomal dominant mutations in *LDLR* gene, gene for its ligand *APOB*, or *PCSK9*. Phenotypic expression of FH can also be caused by variants in the LDL-receptor adapter protein (*LDLRAP*) with recessive inheritance. The objective of our study was to analyze the type and frequency of mutations in these genes in Bulgarian cohort, using whole exome sequencing (WES).

Material and methods: We extracted data for these genes from sequencing files of 604 Bulgarians, analyzed by WES.

Results: We established pathogenic variants, as follows: 3 in *LDLR* (frequency 1:151) - c.1618G>A, c.1646G>A and c.2403_2406del; 2 in *APOB* (1:302) - c.904+2T>C and c.10580G>A; 1 in *PCSK9* - c.1522C>G and *LDLRAP* - c.89-1G>C (1:604). In addition, variants of unknown significance were also found: 8 in *LDLR* (1:75), 29 in *APOB* (1:21), 22 in *PCSK9* (1:28) and 4 in *LDLRAP* (1:151).

Conclusion: The incidence of FH in Bulgaria seems to be higher that the classical population data for 1:500. Currently, there are high-standard identification tests and several lipid-lowering treatments available, most at very low cost (statins), so FH screening should become a priority in health systems promoting public cardiovascular health. Genetic testing of FH provides a reliable molecular diagnosis of FH, prognostic information and the ability to perform precise risk stratification for coronary arterial disease in these patients.

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P07.052.A Exome sequencing identifies pathogenic variants in patients with Atherosclerosis, regardless of risk groups

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Background: Genetic background significantly influences susceptibility to atherosclerosis (AS). We aimed to identify genetic variants in lipid and glucose metabolism genes in Kazakhstani patients with AS.

Material and methods: We performed whole exome sequencing in patients with AS (n = 297) with CHD, arterial hypertension and dyslipidemia. Phenotypic distribution into risk groups was based on the 2019 ESC/EAS Guidelines: very high risk (n = 166), high risk (n = 70), low risk (n = 61). Genetic variants in 17 genes related to lipid and glucose metabolism (*APOE, APOB, PNPLA/2/3, GCKR, TM6SF2, LYPLAL1, ABCB1, ABCG5/8, LDLRAP1, LIPA, LPA, PCSK9, SORT1*) were analyzed in risk groups of patients.

Results: Overall, 2 pathogenic (P), 3 likely pathogenic (LP) and 100 variants of uncertain significance (VUS) were identified in all groups. We found P variants *APOE* (C526T:p.R176C), *GCKR* (G1004A:p.W335X) and LP variants *LDLR* (C419T:p.P140L), *APOE* (T388C:p.C130R), *GCKR* (G307A:p.V103M) in low-risk group patients, P variant *APOE* (C526T:p.R176C) and LP variants *APOE* (T388C:p.C130R), *GCKR* (G307A:p.V103M) in high-risk group patients, P variants *APOE* (C526T:p.R176C) and LP variants *LDLR* (C419T:p.P140L), *APOE* (T388C:p.C130R), *GCKR* (G307A:p.V103M) in the very high risk group.

Conclusion: Pathogenic and likely pathogenic genetic variants were identified independently in all risk groups, mainly in 3 genes (*APOE, LDLR, GCKR*), which emphasizes the need for further studies to assess their clinical significance in pathogenesis of AS. Large number of VUS emphasizes the importance of functional studies to clarify their potential role. Further analysis including other genes involved in metabolic pathways of atherosclerosis development is crucial to deepen our understanding and improve prevention and treatment.

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P07.053.B The impact of chromosomal microarray in congenital heart defects: a single center experience

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Background: Congenital heart disease (CHD) ranks among the most prevalent congenital anomalies and its mortality rate remains significantly high despite various treatment options. This study aims to investigate the diagnostic capabilities and clinical utility of chromosomal microarray (CMA) testing for patients diagnosed with CHD in our center.

Material and methods: A total of 113 patients with CHD were evaluated and divided into two groups: isolated CHD, which included 27 patients, and syndromic CHD, comprising 86 patients. The syndromic CHD group displayed at least one of the following traits: other congenital anomalies, dysmorphic features, intellectual disabilities, or developmental delays. The CMA test was performed on all patients.

Results: In the isolated CHD group, two patients had pathogenic CNVs, and three had variants of uncertain significance (VUS), resulting in a diagnostic rate of approximately 7%. In the syndromic CHD group, ten patients had pathogenic CNVs, and five had VUS, yielding a diagnostic rate of about 11.6%. Within this group, three pathogenic CNVs were classified as large chromosomal aberrations (3.4%). Overall, CMA clarified the molecular diagnosis for 10.6% of patients.

Conclusion: Our results indicated that CMA testing demonstrated a higher diagnostic rate for syndromic CHD compared to isolated CHD, aligning with existing literature. Recurrent microdeletion and duplication syndromes were identified in isolated CHD, whereas CNVs featuring unique breakpoints were also observed in syndromic CHD.

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P07.054.C Increasing the diagnostic yield for Cardiomyopathy (CM) patients: investigating the role of rare Loss of Function (LoF) variants in CM genes for which LoF is not an established disease mechanism

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Background: Next Generation Sequencing (NGS) based gene-panel analysis in CM patients results in a diagnostic yield of ~30%. However, the potential yield is higher because Variants of Unknown Significance yet remain unsolved. The aim of this study is to investigate the role of LoF variants in CM related genes for which LoF is not an established disease mechanism, to potentially increase the diagnostic yield.

Material and methods: We selected the *ACTC1, ACTN2, DSC2, DSG2, MYH7, NEXN, RBM20, TNNI3* and *TNNT2* genes as potentially interesting candidates. We collected LoF variants identified in these genes after NGS-based CM gene panel analysis and (re) evaluated up-to-date molecular and clinical data from literature and (disease) databases, pathogenicity prediction tools and our local patient databases (*i.e.* co-segregation, multiple affected individuals with same variant).

Results: In our cohort of >6300 CM patients, we identified ~35 LoF variants (25 frame shift/nonsense and ~10 affecting RNA splicing (putatively) resulting in LoF) in the above mentioned genes in 120 patients. Clinical and molecular data indicate that

LoF variants in (a subset of) these genes are related to CM phenotype(s).

Conclusion: Our study shows that LoF variants in a subset of CM genes, for which this was not yet established, are involved in disease development, resulting in higher diagnostic yield. We are currently collecting data of other Dutch genome diagnostic laboratories to complement our study. That data and possibly data via other (inter)national collaborations is needed to definitely establish the role of LoF variant in these genes in CM phenotype(s).

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P07.055.D Genetic analysis of sudden cardiac death cases

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Background: Sudden cardiac death (SCD) is a devastating event characterized by an unexpected natural death from cardiac causes. Genetic testing in SCD cases represents a crucial diagnostic tool for identifying inherited cardiac conditions and preventing future tragic events in families. Testing typically focuses on genes associated with cardiomyopathies and channelopathies, which are common causes of SCD, particularly in young individuals.

Material and methods: We utilized a custom capture panel sequencing approach for variant detection and copy number variant analysis in genes associated with inherited cardiac diseases.

Results: We present the genetic findings in a cohort of 395 Czech patients with suspected cardiomyopathies and arrhythmogenic syndromes. Pathogenic or likely pathogenic variants were identified in 104 unrelated patients. Within this cohort, we analysed a subgroup of 52 individuals who experienced sudden death before the age of 40. A pathogenic variant was identified in one case, while variants of uncertain significance were detected in seven cases. In one case, we identified a highly suspected causal variant in the *ACTN2* gene. Segregation analysis revealed that the proband's father carries this variant in a mosaic pattern, with the variant present in 24% of leukocytes, yet he remains asymptomatic and free of cardiac abnormalities.

Conclusion: In conclusion, our findings demonstrate the challenges and complexities of genetic testing in SCD cases. Although the detection rate of pathogenic variants was relatively low, the identification of a mosaic carrier parent highlights the difficulties in interpreting genetic findings and their clinical relevance.

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P07.056.A Evaluation of a hereditary haemorrhagic telangiectasia specialist clinic in Scotland

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Background: Hereditary haemorrhagic telangiectasia(HHT) is a rare, multisystem genetic disorder that requires management of arteriovenous malformations(AVMs), epistaxis, and anaemia. We evaluated the HHT specialist clinic that was established in NHS Tayside in 2020.

Material and methods: HHT clinic data from 2020–2025 was reviewed. Linear mixed model regression was used to assess correlation between Quality of Life(QoL), haemoglobin(Hb), Epistaxis Severity Score(ESS) and time attending clinic.

Results: The 71 patients had a median age of 55.5years(IQR 31.3–70.5years), with a median of 6 appointments(IQR 3–11). Pathogenic Variants were identified in 66; 68%(45/66) ACVRL1; 24%(16/66) ENG; 8%(5/66) SMAD4. 32%(23/71) were screened for brain AVMs, with one detected(4%,1/23). 86%(61/71) were screened for pulmonary AVMs(PAVMs), with a prevalence of 30% (18/61). PAVM prevalence differed by genotype ($p < 0.01$); *ENG* carriers had higher PAVM prevalence than *ACVRL* carriers (58%(7/12) vs 15%(6/40), $p = 0.023$). 60%(3/5)*SMAD4* carriers had PAVMs.41%(29/71) received intranasal procedure(s), with 72% (21/29) requiring multiple procedures. 44%(31/71) are currently receiving iron replacement therapy, with 8%(6/71) requiring blood transfusions. 6%(4/71) have received bevacizumab treatment. QoL scores were positively correlated with Hb levels(beta0.22, $p < 0.01$) and negatively correlated with ESS(-2.98units, $p < 0.001$). After 6-months of clinic intervention ESS had decreased (-0.56,$p = 0.04$), but had returned to baseline by 2-years. There were no significant changes in QoL or Hb levels over 2-year follow-up.

Conclusion: The high intervention levels - for intranasal procedures, iron and/or blood transfusions and PAVM embolisation - support the need for a specialist service. The correlation between QoL and Hb/ESS suggests that a focus on more effective interventions to control epistaxis is required.

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P07.057.B Genetic insights into pediatric cardiac disorders: results from a Cohort study of 102 patients

Maria Luca[1], *Angelo Corso Faini*[1], *Francesca Arruga*[2], *Fiorenza Mioli*[1], *Claudia Saglia*[1], *Giulia Margherita Brach Del Prever*[1], *Carmelo Maria Romeo*[1], *Caterina Scolari*[1], *Tullia Carradori*[1], *Fulvio Gabbarini*[3], *Chiara Riggi*[3], *Francesca Cairello*[4], *Tiziana Vaisitti*[1,2], *Alessandro Mussa*[5,6], *Diana Carli*[1,2], *Silvia Deaglio*[1,2]

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Background: Next-generation sequencing (NGS) is routinely used for pediatric arrhythmias, cardiomyopathies, and collagenopathies and is increasingly applied to congenital heart defects. This study reports on 102 pediatric patients across these four categories.

Material and methods: Targeted in silico NGS and phenotype-driven clinical exome sequencing (CES) in negative cases were followed by parental segregation with Sanger sequencing.

Results: Overall, 37/102 (36%) patients tested positive with heterogenous diagnostic yields across subcategories: arrhythmic syndromes showed the highest rate (13/25,52%), followed by cardiomyopathies (7/21,33%), collagenopathies (3/9,33%) and congenital heart defects (14/47,30%). Right-sided obstructive lesions had the highest diagnostic rate (10/17,59%), with variants in RASopathy-genes, *GATA4*, *ELN*, and *JAG1*, followed by isolated septal defects (2/5,40%), including a familial *NKX2.5* case. Among cardiomyopathies, two myopericarditis cases harbored *DSP* variants, highlighting the importance of genetic testing in inflammatory presentations. Overall, variants of uncertain significance (VUS) were 14/102 (14%). Notably, a VUS in *MCTP2* segregated in a family with aortic coarctation, supporting its potential role in left-sided obstructive lesions. Unexpected findings included *MYBPC3* and *PKP2* pathogenic variants in two collagenopathies patients diagnosed respectively with 4q12q21 microdeletion syndrome and *CACNA1C*-related Timothy syndrome. Phenotype-driven CES analysis enabled additional diagnoses, including a pathogenic *WDFY3* variant in an autistic patient with aortic diameter at the upper limit.

Conclusion: Diagnostic yield in this study aligns with literature data, confirming the growing relevance of genetic testing in pediatric cardiology, both for isolated and syndromic presentations. Beyond confirming known associations, genetic testing refines clinical diagnoses, uncovers unexpected findings, guides risk stratification and personalized management.

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P07.058.C A case report of a patient affected with facial CM-AVM caused by two somatic RASA1 mutations

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Background: Vascular malformations are associated with a heterogeneous group of diseases including mosaic disorders caused by post-zygotic somatic mutations. Mosaic diseases require specialized diagnostic methods to detect the causative somatic variant. A patient with a large facial capillary-arteriovenous-malformation (CM-AVM) was referred for genetic testing. Previous testing of affected tissue in pathology had identified a single likely pathogenic somatic variant in *RASA1*, but no second hit. *RASA1* has previously been described in CM-AVM with a combination of heterozygous germline variants plus somatic second hit mutations.

Material and methods: We performed deep exome sequencing using unique molecular identifiers of DNA from both healthy and affected tissue. The data was processed and uploaded to a local version of the cBioPortal software, an open access platform originally designed for cancer genomics.

Results: We were able to identify two somatic variants in *RASA1*. The previously described variant c.1698+2T>C with a variant allele fraction (VAF) of 7% and the second variant c.2603+1G>A with a VAF of 4%. Both variants are predicted to affect splicing and have previously been classified as (likely) pathogenic in ClinVar. We therefore present the disease-causing combination of two somatic variants in RASA1 in a patient with a large facial CM-AVM.

Conclusion: Integrating diagnostic strategies from cancer diagnostics into the evaluation of mosaic vascular malformations can significantly improve the diagnostic yield.

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P07.059.D Phenocopies in Brugada Syndrome: do they really look similar?

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Background: Brugada syndrome (BrS) is a cardiac arrhythmia disorder with an increased risk of sudden cardiac death, primarily linked to pathogenic variants in the *SCN5A* gene. Growing evidence suggests significant genetic heterogeneity, with variants in other genes potentially contributing to similar clinical manifestations. Furthermore, phenocopies represent a key challenge in differential diagnosis.

Material and Methods: WES on 87 individuals from 70 families with suspected BrS.

Results: Sixteen exhibited variants in genes not classically associated with BrS, suggesting a broader genetic spectrum underlying BrS-like phenotypes. Among them, five members of a family (including a mother with an implanted defibrillator and a sister with two episodes of cardiac arrest) harboured a 1q43 duplication, disrupting *RYR2*—an alteration undetectable by WES. Two patients (father and son) carried a variant in *DES*. Another patient had a variant in *LMNA* without signs of dilated cardiomyopathy and a *TRPM4* variant. Interestingly, *TRPM4* was also mutated in another family with a BrS diagnosis. In one family, BrS segregated with congenital heart defects, ranging from tetralogy of Fallot to transposition of the great arteries, with variants in *GDF1* and *SCN1B*, either together or separately. Surprisingly, one individual carried a hypomorphic homozygous variant in *CLN3*, the only form of neuronal ceroid lipofuscinosis known to involve cardiac manifestations. Deep phenotyping revealed early neurodegenerative symptoms at age 52.

Conclusion: These findings confirm that other known genes may contribute to BrS-like manifestations and phenocopies. Integrating comprehensive genomic analysis and deep phenotyping is essential for precise diagnosis and risk stratification,

ultimately guiding personalised therapeutic approaches in arrhythmia syndromes.

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P07.060.A Blood DNA methylation signatures for more common forms of pulmonary hypertension

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Background: Pulmonary hypertension (PH) describes conditions characterised by increased blood pressure in the pulmonary artery and right heart, leading to premature death by heart failure. Epigenetic variation in PH broaches the potential for pharmacological targets and a means by which to understand disease penetrance. Our recent pilot study (Ulrich, 2024, PMID: 38184627) from the UK PAH cohort established three significantly associated epigenomic loci hypermethylated in pulmonary arterial hypertension (PAH) (*CTSZ*, *COG6*, and *ZNF678*). Our study aims to validate and build upon these findings by conducting an epigenome-wide association study (EWAS) on blood DNA methylation data from individuals with PH of more common aetiologies alongside PAH, and performing a meta-analysis combined with pilot data.

Material and methods: We performed quality control (QC) on new IlluminaEPICv2.0 methylation data for 375 individuals (415 before QC): 116 disease-free controls, 195 patients under five PH diagnostic subgroups, and 64 PAH patients. We used the CPACOR pipeline (Lehne, 2015, PMID: 25853392) and the GLINT command-line tool to pre-process the raw methylation data, and EpiSmokEr to infer smoking status. Alongside the pilot study, total number of cases post-QC including all individuals with PH is 688, with 1,242 controls.

Results: We will conduct a case-control analysis combining the total sample data in a meta-analysis and investigate potentially discernible differences in methylation patterns between PH subtypes. We will present the results of our meta-analysis of these two studies.

Conclusion: By expanding the PH analysis, we will produce robust EWAS expanding the knowledge about molecular signatures of this rare condition.

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P07.061.B Evaluation of genetic variants in Kazakhstani patients with atrial fibrillation: insights from targeted next-generation sequencing

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Background: Atrial fibrillation (AF) is the most common type of cardiac arrhythmia. AF affects over 33 million people globally, with a 25% lifetime risk. AF causes an irregular, often rapid heartbeat and raises the risk of heart failure, stroke and cardiac death. We aimed to evaluate the mutation spectrum of patients diagnosed with AF by targeted next-generation sequencing (NGS) in Kazakhstan.

Material and methods: We performed deep-coverage targeted NGS of gDNA isolated from whole blood samples of 47 AF patients on Illumina HiSeq 2000 platform. A designed custom HaloPlex panel of 96 genes associated with inherited cardiac arrhythmias and cardiomyopathies was used in our study. SureCall 2.0.7.0 software was applied for bioinformatics analysis. The clinical significance of obtained variants was interpreted according to ACMG guidelines.

Results: Genetic screening revealed 55 rare variants. We found 6 pathogenic (P) and 8 likely pathogenic (LP) variants in 14 (29.8%) individuals. Namely, disease-causing mutations are detected in *MYH7, CACNB2, LAMA2*, and *TTN* genes. In addition, 41 variants of unknown significance (VUS) with suggestive evidence of pathogenicity were identified in 28 (59.6%) patients. Moreover, pathogenic variants by functional characteristics were most frequently found in genes encoding sarcomeres (31%), ion channels (11%), and cell membranes (9%).

Conclusion: The study revealed clinically significant variants within 96 genes associated with cardiac diseases. We identified rare P/LP variants suspected to cause AF in 29.8% of patients showing a higher predisposition to severe phenotypes. Besides, a high occurrence of VUS has been observed, highlighting the need for functional studies.

C.O.I. Disclosure:

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P07.062.C Rare pathogenic variant *KCNH2*c.1825_1830del can lead to arrhythmia LQTS type 2 with consciousness loss episodes, multiple craniofacial and skeletal anomalies

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Background: Seizures like episodes activity is well-known reason for physical and intellectual delay leading to sudden death. Significant clinical and genetic heterogeneity of seizures poses a serious challenge for early diagnosis, and only molecular genetic testing helps to solve a problem in time. Aim of our study: Search for genetic cause of regular loss of consciousness in 19-year-old man with uncertain multiple craniofacial and skeletal anomalies.

Methods: Clinical genetic testing, sequence analysis and deletion/duplication testing of the 2095 genes ("Invitae").

Results: At the age of 13, a patient began to lose consciousness, sometimes with episodes of severe abdominal pain. He lost weight and began to lag of height and physical activity. Clinical examination revealed numerous craniofacial and skeletal anomalies: trigonocephaly, triangular flat face, disproportionately wide neck, microstomia, microretrognathia, deformation of distal phalanges I, III and V hand's fingers, keeled chest deformity, scoliosis. The patient had autistic behavior and was treated as an epileptic. Rare pathogenic variant *KCNH2* c.1825_1830del(p.Asp609_Lys610del) was identified. It's

important, that *KCNH2* gene is associated with autosomal dominant long QT syndrome (LQTS), type 2, which may result in syncope and seizure-like episodes activity (MedGen UID: 462293). After 4-day outpatient Holter recording the cardiologist's conclusion was coincident with molecular testing: Congenital long QTS syndrome type 2; paroxysmal form of pirouette ventricular tachycardia with hemodynamic disturbances and syncope. The patient was directed for implantation of a cardioverter-defibrillator.

Conclusion: Genetic testing confirmed the pathogenicity of the KCNH2c.1825_1830del variant as a cause of seizure-like episodes activity due to LQTS type 2 arrhythmia.

C.O.I. Disclosure:

H. Akopyan: None. **K. Bakum:** None. **N. Helner:** None.

P07.063.D A rare case of hypertrophic-restrictive cardiomyopathy with extensive myocardial and valvular calcifications: whole-exome sequencing (WES) analysis of an uncommon clinical phenomenon

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Background: Myocardial calcifications are rare, typically resulting from acquired factors. A small proportion of cases are monogenic disorders, and genes such as *ABCC6* and *ENPP1*, which are an established cause of arterial calcifications, have recently been associated.

Material and methods: A 57-year-old woman underwent cardiological evaluation, echocardiography, cardiac-magnetic-resonance (cMR), myocardial biopsy, and WES.

Results: The patient, evaluated for exertional dyspnoea, underwent echocardiography that revealed mitral annular calcifications. cMR showed diffuse intra-myocardial calcifications, at midwall and subendocardial layers, associated with hypertrophy, suggesting hypertrophic-restrictive cardiomyopathy. Myocardial biopsy suggested a metabolic-driven cardiomyopathy. WES identified two compound heterozygous variants in the *ENPP1* gene (NM_006208.3), which encodes ectonucleotide pyrophosphatase/phosphodiesterase 1, regulating extracellular pyrophosphate and calcium phosphate homeostasis: a pathogenic splicing variant (ACMG) c.2311+1G>A and a missense variant c.2330A>G p.(H777R), classified as VUS (ACMG). Segregation analysis in the healthy siblings showed that two of them carried one variant each. *ENPP1* variants are a well-known cause of arterial calcifications, ranging from severe early-onset to milder, later-onset forms. In severe cases, variants cluster in functional regions, though no clear genotype-phenotype correlation is found. While primarily linked to arterial and valvular calcifications, myocardial calcifications may represent a rare, poorly characterized phenotypic expression.

Conclusion: The identification of biallelic *ENPP1* variants highlights the role of genetic testing in this condition, underlining its relevance for recurrence risk assessment and personalized treatment. Indeed, bisphosphonates might be a valuable option, though patients' outcome have been variable (PMID: 29921207). A functional proteomic study is assessing the variants' impact, considering innovative enzyme replacement therapies (PMID: 39342427).

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P07.065.B Investigating sudden death in two sisters: the role of molecular autopsy

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Background: Sudden cardiac death (SCD) poses a significant risk in children and young adults, frequently linked to genetic predispositions. Molecular autopsy (MA) refers to DNA-based identification of the cause of death. This case study explores the genetic underpinnings of sudden death in two sisters.

Material and methods: The proband, a 14-year-old female who suddenly died at home, was the third child to a healthy couple. Her 16-years-old sister deceased 17 years prior while bathing in the sea. MA on the proband and parents DNA was performed by whole exome sequencing (WES). Sampling with forensic methods was done on objects belonging to the first sister and genomic DNA was obtained by swabbing a trouser. Both parents and the second child of the couple have normal cardiologic investigations.

Results: WES of the proband identified the paternally inherited pathogenic heterozygous c.7175A>G (p.Tyr2392Cys) variant in *RYR2* gene, cause of catecholaminergic polymorphic ventricular tachycardia (CPVT) characterized by arrhythmias during physical stress. Minisequencing confirmed the same RYR2 variant in the first sister suggesting this variant contributed to their sudden deaths. The presence of the same variant in the healthy father is an unexpected finding as mean penetrance of RYR2 pathogenic variants is high (>80%).

Conclusion: MA provided a crucial explanation for the family and allowed for accurate risk assessment and genetic counseling for the surviving sister. This case underscores the importance of MA in sudden cardiac death even in the absence of family history advocating its implementation as a standard practice in unexplained fatalities.

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P07.066.C Mutational burden of genetic variants in cardiomyopathy-associated genes provides new insights for postmortem molecular diagnosis of SCD in Kazakhstan

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Background: The study aimed to evaluate the mutational burden in Kazakhstani Sudden cardiac death (SCD) young (<45 yo) victims revealed by next-generation sequencing.

Material and methods: Targeted sequencing of 174 candidate genes associated with heart diseases was conducted in 67 SCD victims. Based on forensic examination results, SCD cases were divided into three groups: coronary artery disease (CAD) postmortem SCD/CAD (n = 28), non-diagnostic cardiac changes and secondary cardiomyopathy postmortem SCD/SCM (n = 34), and SCD with other heart anomalies (n = 5).

Results: We identified 537 unique variants among all cases. According to ACMG, three variants were categorized as pathogenic (P), 8 as likely pathogenic (LP), and 179 as variants of uncertain significance (VUS). Rare pathogenic variants were identified in 11 out of 51 cases, including 3 (27%) individuals who died from CAD. The corresponding frequency among SCM group was 64%. Comparative analysis of data between groups of SCD/CAD, SCD/SCM, and the control group of 78 people without CVD has shown that 1) the number of homozygous variants was predominant in the SCD/CAD group ($p = 0.0514$); 2) the number of rare variants in both groups was relatively similar; however, the distribution of variants in the SCD/SCM group differs significantly ($p = 0.0023$); 3) unique variants predominates in those who died from SCD ($p = 0.0002$), particularly in the SCD/SCM group; 4) the mutational burden in the SCD/SCM group predominates compared to those who died from SCD with CAD ($p = 0.0182$).

Conclusions: Increased genetic variant burden (P and VUS) is associated with non-diagnostic cardiac changes but not CAD.

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P07.067.D Influence of genetic factors on the severity of monogenic familial hypercholesterolemia

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Background: Monogenic familial hypercholesterolemia (FH) is one the most common hereditary disease characterized by an increased level of low-density lipoprotein cholesterol (LDL-C) and risk of atherosclerotic cardiovascular disease (ACVD). FH has variable expressivity that can be influenced by other genetic factors. The aim of the study is to determine the prevalence of co-existence and influence of LDL-C polygenic score (PS) and high level of Lp(a) (Lp(a)>50 mg/dL) on the severity of FH.

Material and methods: The study included 27 genetically confirmed «definite» and «probable» FH patients. All patients underwent electrophoresis of lipoproteins, measurement of Lp(a) and PS.

Results: The frequency of pathogenic variants in *LDLR* and *APOB* genes was 72.1% and 25.9%, respectively. The most common variant in *LDLR* gene was p.Glu101Lys (n = 6), in *APOB* gene - p.Arg3527Gln (n = 6). Large deletions and duplications in *LDLR* gene were not identified. Electrophoresis showed type IIa and IIb pattern of WHO classification of hypercholesterolemia in 90.9% and 9.1% of cases, respectively. High levels of Lp(a) was detected in 42.9% of patients. High PS (PS ≥ 0,8) was detected in 26.1% of cases. Pathogenic variants in the *LDLR* and *APOB* genes, high PS and high level of Lp(a) were detected in 15.4% of patients. These patients were characterized by higher incidence of ACVD ($p = 0.036$) and higher level of LDL-C compared to FH (9.80 vs 6.97 mmol/L, $p = 0.039$).

Conclusion: Monogenetic FH, high PS and high level of Lp(a) can co-exist in patients with FM. High PS and high level of Lp(a) influence the severity of FM.

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P07.068.A Novel *LRP1*variant in a patient with idiopathic pulmonary arterial hypertension

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Background: Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by the remodeling of the pulmonary arterial vasculature. It can cause heart failure and death if untreated. In 2000, the *BMPR2* gene was the first gene related to PAH. Since then, the development of next-generation sequencing has allowed us to identify 27 new genes associated with the disease.

Material and methods: Whole exome sequencing was performed in a patient with idiopathic PAH who had a negative result on the PAH gene panel.

Results: We detected a heterozygous missense variant (c.8185C>T) in the *LRP1* gene. This gene codifies a receptor involved in different pathways, like vascular homeostasis. LRP1 regulates the TGF-β signaling pathway, the most studied one in PAH, by interacting with TGF-β family receptors. According to some studies, LRP1 is overexpressed in lung and smooth muscle cell (SMC) samples from PAH patients. However, there are reports of *LRP1* knockout SMC in mice that spontaneously develop this pathology. It is believed that the elevated protein levels in PAH patients may be due to compensatory mechanisms in response to PAH conditions since this protein is involved in tissue homeostasis.

Conclusion: According to that, we hypothesize that this variant, which affects an interaction domain, could cause a loss of function of LRP1 and consequently upregulate the TGF-β pathway, which leads to typical PAH pathobiological changes. This study adds genetic evidence about the relationship between *LRP1* and PAH development.

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P07.069.B A novel *ACTC1* gene mutation in a family with left ventricular noncompaction

Kinga Hadzsiev[1], *Peter Nagy*[2], *Reka Faludi*[3], *Gergely Buki*[1], *Kira Dorogi*[1], *Agnes Till*[1], *Renata Szalai*[1]

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Background: Alpha cardiac actin (ACTC1) is the major protein of cardiac sarcomeric thin filaments and is essential for cardiac muscle contraction. Mutation in the *ACTC1* gene has been associated with left ventricular noncompaction, hypertrophic and dilated cardiomyopathy, and atrial septal defects. These overlapping allelic disorders show high variability in severity.

Material and methods: A four-month-old female patient phenotypically characterized by a not uneventful perinatal period, cardiomegaly, and echocardiography identified left ventricular non-compact cardiomyopathy. Due to severe heart failure, she underwent a heart transplantation at age of 10 months, as a result her condition and psychomotor development are improved. Based on clinical findings a multigene NGS cardiomyopathy panel test was performed.

Results: NGS panel analysis detected a heterozygous c.43G>C (p.Gly15Arg) variant in *ACTC1* gene. The variant was classified with uncertain significance followed by ACMG guideline, so parental carrier testing was carried out. The presence of the variant was confirmed in the DNA sample of the asymptomatic father. As the child's symptoms were consistent with the left ventricular noncompaction and dilated cardiomyopathy phenotypes, and the variant was inherited from the father, father was referred to cardiology examination, which detected marked left ventricular hypertrabeculation.

Conclusion: The implementation of intrafamilial carrier testing allows for the appropriate classification of variants of uncertain pathogenicity. Furthermore, in some cases, with the knowledge of the presence of a mutation detected in an asymptomatic state, which may later manifest as a severe disease, with an early prevention and follow-up can significantly improve the quality of life and lifespan.

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P07.070.C A shield against Brugada: the p.His558Arg polymorphism in action

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Background: Brugada syndrome, an autosomal dominant inherited channelopathy, predisposes individuals to arrhythmias and sudden cardiac death. Pathogenic *SCN5A* variants account for 20–30% of Brugada syndrome cases, with reduced penetrance and variable expressivity.

Clinical Report: A 55-year-old female initially referred for evaluation of pheochromocytoma, a malignancy with a 40% association with hereditary cancer syndromes. Clinical exome sequencing identified a pathogenic *SCN5A* variant (c.845G>A; p.Arg282His) along with a potentially protective polymorphism (c.1673A>G; p.His558Arg). While incidental *SCN5A* variant raised concerns about Brugada syndrome, the patient demonstrated no phenotypic or electrocardiographic signs, even under conditions that could provoke arrhythmias, such as electroconvulsive therapy for psychosis and adrenergic stress. Ajmaline provocation testing revealed no diagnostic features of Brugada syndrome. A detailed review of the literature revealed that p.His558Arg can partially compensate for sodium channel dysfunction caused by pathogenic *SCN5A* variants, potentially explaining the patient's asymptomatic presentation.

Discussion: This case highlights three critical considerations: (1) reduced penetrance in genetic channelopathies may be modulated by variant interactions, emphasizing the need to explore compensatory mechanisms; (2) filtering variants based solely on allele frequency may overlook clinically significant protective modifiers; (3) despite the patient's asymptomatic status, the co-inheritance of pathogenic *SCN5A* variants with protective polymorphisms warrants familial genetic screening due to a 50% risk of transmission and the potentially fatal nature of Brugada syndrome.

Conclusion: This case underscores the importance of integrating molecular findings with clinical evaluation and illustrates how personalized medicine approaches can guide risk assessment, therapeutic decisions, and familial screening in inherited cardiac syndromes. **Grants**: None.

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P07.072.A *ABCA3* and *NOTCH1* mRNA expressions differ in chronic thromboembolic pulmonary hypertension patients with rare missense variants

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Background: Pulmonary hypertension (PH) is characterized by elevated blood pressure in the pulmonary arteries, eventually damaging the right heart. Chronic thromboembolic PH (CTEPH) is a rare subtype of PH due to persistent obstructive thrombi with undefined genetic origins. This study aims to analyze the expression levels of the genes in which CTEPH patients have genetic variants.

Material and methods: DNA and RNA samples were isolated from blood samples of 15 CTEPH patients. WES was conducted on the Illumina NovaSeqX instrument using Sureselect V6 kit. A total of 41 PH-related genes were examined. The mRNA expression levels were determined using qRT-PCR and statistically analyzed.

Results: Among 15 CTEPH patients, 4 unique VUS were found in *ABCA3* (1 missense) and *NOTCH1* genes (2 missense, 1 splice site) in 4 patients. A 45% decrease in *ABCA3* expression was observed in the patient with *ABCA3* (NM_001089.3:c.1304G>A) variant, while *NOTCH1* expression was increased by 43% in the patient with *NOTCH1* (NM_017617.5:c.4692T>G) variant compared to others. Moreover, the 6-minute walking distance was shorter for the patients with rare *NOTCH1* variants than other CTEPH patients ($p = 0.009$). Additionally, patients with *NOTCH1* variants had higher New York Heart Association functional classes, indicating more severe heart failure ($p = 0.014$).

Conclusion: The findings suggest that rare *ABCA3* and *NOTCH1* variants alter the mRNA expression levels of the genes. The pathophysiology of CTEPH is rooted in thromboembolic events; however, the genetic alterations governing proliferation within the pulmonary vasculature may predispose individuals to PH and correlate with more severe manifestations of the disease.

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P07.073.B Noncompaction cardiomyopathy, sinus node dysfunction and aortic dilatation in family with HCN4 variant

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Background: HCN4 pathogenic variants cause bradycardia and sinus node dysfunction (SND). There is increasing reports of a more complex phenotype, including noncompaction-cardiomyopathy, aortic dilatation (AD), mitral valve defects, early-onset atrial fibrillation (AF), atrioventricular block and ventricular-tachycardia, caused by pathogenic variants in a specific domain of *HCN4*.

Material and methods: We evaluated a 47-year-old woman and her 18-year-old son, both with bradycardia and AD. The woman also has hypertrophic cardiomyopathy (HCM) and AF, suspected for Marfan-like-syndrome without eye/skeletal involvement. Her son also had mitral valve prolapse and left ventricular noncompaction (LVNC). Family history revealed cardiomyopathy, bradycardia, aortic dilatation and HCM.

Results: Multi-gene-exome-based-panel testing revealed a likely pathogenic variant in *HCN4* (c.1456C>T, p.Pro486Ser) in the mother, and excluded *FBN1* and other connective-tissue genes variations as a cause. Her son was found heterozygous for the same variant.

Conclusion: *HCN4* variant, usually associated with SND, was found in a family with a more complex phenotype of LVNC and AD, mitral valve defects and early-onset AF. The p.Pro486Ser is very rare and located just upstream to transmembrane-domain-6, a location characteristic of other variants previously reported to cause this phenotype. This further confirms the existence of a specific *HCN4* region associated with the combined phenotype of sinus bradycardia, LVNC, and thoracic aortic aneurysms. Another variant near this region, A485V, a known Moroccan-Jews founder, was originally reported in 2010 as causing SND. More recently it has been reported to cause AF and LVNC. Based on these reports, we believe that A845V heterozygotes should be screened for AD.

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P07.074.A Identification of a clinically relevant *MYBPC3* copy number variation using whole-Gene NGS sequencing

Laura Cazon[1], *Noël Brögger*[1], *Marlene Pérez*[1], *Iria Gómez*[1], *Rosalía Debén*[2], *María Sánchez*[1], *Xusto Fernández*[1], *Diego Cabrera*[1], *Almudena Amor*[1], *María Valverde*[1], *Soledad García*[1,3], *Ivonne Cárdenas*[1], *Martín Ortíz*[1], *Juan Pablo Ochoa*[1,4]

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Background: Pathogenic variants in *MYBPC3* are a well-established cause of hypertrophic cardiomyopathy (HCM), with loss-of-function variants typically associated with this phenotype. Haploinsufficiency is considered the primary disease mechanism. However, copy number variations (CNVs) in *MYBPC3* are rare and not routinely screened for in clinical settings. Here, we report a novel *MYBPC3* CNV in a 53-year-old male proband with dilated cardiomyopathy (DCM), an atypical presentation for loss-of-function variants in this gene, and a family history of HCM and sudden cardiac death.

Material and methods: The patient's genomic DNA was analyzed using a next-generation sequencing (NGS) panel covering the entire *MYBPC3* gene. CNVs were detected via a read-depth approach and validated by orthogonal molecular techniques (Sanger sequencing).

Results: A novel 17 kb deletion encompassing exons 13–35 and the 3′ UTR of *MYBPC3*, as well as part of the 3′ UTR of *MADD*, was identified. This variant was classified as likely pathogenic (LP) according to ACMG guidelines and ClinGen CNV classification criteria.

Conclusion: This case presents an unexpected phenotype, as DCM is not the typical manifestation of *MYBPC3* loss-of-function variants. The finding highlights the need for further investigation into the phenotypic variability associated with *MYBPC3* CNVs. Additionally, it underscores the importance of comprehensive *MYBPC3* NGS sequencing, including CNV detection, to enhance molecular diagnosis in patients with cardiomyopathies.

C.O.I. Disclosure:

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P07.075.D Neonatal Marfan syndrome: a spontaneous mutation necessitating cardiac intervention

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Background: Neonatal Marfan syndrome (nMFS) can arise from a spontaneous pathogenic variant in the FBN1 gene. Genotype-phenotype correlations have associated this rare MFS subclass with specific 24–34 exon region (NM_000138.5) pathogenic variants. nMFS is associated with a more severe phenotype than the classical autosomal dominantly inherited Marfan syndrome. Congestive cardiac failure within the first few years of life is the primary cause of mortality in nMFS, with limited research into treatment interventions.

Material and methods: We analysed the genetic phenotypes and efficacy of cardiac treatment interventions in three cases of nMFS

Results: All cases had an FBN1 pathogenic variant between the 25–26 exons (gene transcript ENST00000316623.10)(Table 1). Case 1: At age 12, they were cardiac symptom-free and intervention was limited to an attempted ASD closure. Case 2: underwent mitral and tricuspid valve repair within the first year of life. They subsequently underwent a PEARS procedure at nine years old. At age 14, they were attending school full-time on cardiac pharmacotherapy with recurrent supraventricular tachycardia. Case 3: underwent two major cardiac surgeries. The first was mitral and tricuspid valve repair at two months old, requiring a repeat tricuspid valve repair at 24 months. At age seven, they were attending school full-time with significant skeletal abnormalities and requiring CPAP and BiPAP.

Conclusion: These cases demonstrate that by proactively combining early high-risk cardiac procedures with ongoing cardiovascular pharmacotherapeutics, nMFS patients can be treated to surpass previously reported life expectancies.

Table 1

	Case 1 (exon 25)	Case 2 (exon 26)	Case 3 (exon 26)
FBN1 variant	**c.3032G>T [p.(G1011V)]**	**c.3115T>C [p.(C1039R)]**	**c.3143T>C [p.(I1048T)]**
Cardiovascular Intervention	**At 8 years old:** An unsuccessful attempt at device closure of an atrial septal defect (ASD).	**Within first year of life:** Mitral valve repair (ring annuloplasty) and tricuspid valve repair (de Vega annuloplasty), Patent Foramen Ovale (PFO) left open.**At 9 years old:** Redo sternotomy (second sternotomy), dissection of mediastinal adhesions, aorto bicaval cardiopulmonary bypass, complex mitral valve repair with CE Physio II annuloplasty size 28 mm, personalised external aortic root support (PEARS) procedure to the aortic root and ascending aorta.	**Within first year of life:** Secundum ASD closure, mitral valve repair (chordae shortening and partial annuloplasty), tricuspid valve repair (leaflet repair and partial annuloplasty). Re-do repair of mitral valve followed by mitral valve replacement.**22 months post initial operation:** Prosthesis & Tricuspid valve repair with 6 days of Extracorporeal Membrane Oxygenation post operatively.
Cardiovascular Pharmacotherapy	N/A	atenolol 40 mg twice daily, aspirin 75 mg once daily, digoxin 100 micrograms twice daily, irbesartan 75 mg once daily	bumetanide 0.9 mg three times per day, atenolol 10 mg twice daily, spironolactone 15 mg twice daily, digoxin 70mcg twice daily, irbesartan 90 mg once daily, warfarin, sildenafil 15 mg three times per day
Non-Cardiac Features	**At 12 years:**, hypermobile joints & spine, flat feet, height: >99.6th centile, visual problems, lens dislocation - wearing glasses.	**At 14 years:** severe scoliosis, multiple joint laxity, spinal surgery with extendable rods at 3 years and 8 years of age. lens subluxation, retinal detachment with 2 eye surgeries.	**At 7 years old:** uses a wheelchair, requires continuous positive airway pressure (CPAP) and bilevel positive airway pressure (BiPAP) therapy.

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E. Harte: None. **N. Naqvi:** None. **A. Child:** None. **J. Aragon-Martin:** None.

P07.076.A Diagnostic and prognostic value of whole-exome sequencing in heart transplant donors and recipients

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Background: Advancements in next-generation sequencing have significantly enhanced our understanding of donor-recipient compatibility, the genetic etiology of end-stage organ failure, the influence of genetic polymorphisms on transplant outcomes, and the pharmacogenetics of immunosuppressive therapies [1]. In Kazakhstan, where heart transplantation (HTx) remains rare, there is limited data on the genetic profiles of donors and recipients [2].

Material and methods: An observational study recruited 32 pairs of donors and recipients of HTx over the past 10 years at the "University Medical Center" Corporate Fund (Astana, Kazakhstan). Whole-exome sequencing (WES) was carried out on the NovaSeq 6000 platform.

Results: Pathogenic and likely pathogenic variants were identified in 9 recipients with dilated cardiomyopathy in the TTN, RBM20, LMNA, and MYH7 genes, and in 2 recipients with hypertrophic cardiomyopathy in the MYH7 and MYH7B genes. Variants of uncertain significance were detected in 17 heart recipients, affecting genes such as TTN, TTNI3, NOTCH1, FLNC, LAMA4, CACNA1C, SYNE2, RBM20, PKP2, MYH6, MYBPC3, ACTN2, and TNNC1. Genomic sequencing of donors revealed one pathogenic variant (KCNE1) and one likely pathogenic variant (RYR1) among two heart recipients. The study identified variations in genes associated with immunosuppressive drug metabolism and transport, including CYP3A4, ABCB1, ABCC2, CYP2C19, CYP2C8, CYP2J2, ABCC8, NOD2, NR1I2, POR, SUMO4, and UGT1A8, among heart recipients.

Conclusion: WES identified significant variants in recipients associated with cardiomyopathies and immunosuppressive drug metabolism, as well as in donors linked to sudden cardiac arrest. The demonstrated utility of sequencing in post-transplant management highlights the need for further validation through longitudinal studies.

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P07.077.B Whole exome sequencing as molecular diagnostic tool for congenital heart defects

Valentina Peycheva[1,2], *Kristiyana Vitanova*[1], *Kunka Kamenarova*[1,2], *Kalina Mihova*[1,2], *Darina Kachakova*[1,2], *Martin Georgiev*[1,2], *Olga Slavcheva*[3], *Ivelina Ivanova*[3], *Maria Sredkova-Ruskova*[3], *Anelia Nankova*[4], *Maria Tarneva*[4], *Simeon Shishkov*[5], *Ivanka Dimova*[2,6], *Radka Kaneva*[1,2]

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Background: Development of exome based molecular approaches has led to the identification of numerous genes involved in cardiac morphogenesis and have a role in the pathogenesis of congenital heart diseases (CHD). The aim of this study was to identify the genetic causes of either isolated CHD, or cardiovascular defect in the context of syndromic condition.

Material and Methods: Nine Bulgarian patients with non-syndromic or syndromic CHD were referred to our lab. Whole exome sequencing (WES) using NovaSeq 6000 sequencing platform (Illumina) was performed. In non-syndromic cases a CHD gene panel comprising 546 genes was used for the analysis. Confirmation Sanger sequencing of the discovered variants was performed.

Results: In seven patients an overall of 16 variants of interest were detected. Four pathogenic/likely pathogenic variants and 12 variants with unknown significance (VUS), but with potential role in the disease phenotype were found. Nine of them were identified in genes directly associated with the cardiovascular phenotype of the patients - *DTNA, LDB3, MYH6, MYH7, MYH11, NEBL, TBX1, TMEM43, TTN*. Among them, one likely pathogenic variant in *MYH6* gene and one VUS in *MYH11* gene were novel. Two of the detected variants in *MYH7* and *MYH11* genes confirmed the suspected diagnosis in two patients with family history of cardiovascular defect.

Conclusion: WES is powerful diagnostic tool for detection of disease-causing genes in both syndromic and non- syndromic CHD. This helps to improve the individual therapy and prophylactics to reduce the risk of sudden cardiac death in the affected individuals and to elucidate the recurrent family risk.

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P07.078.C Germline mosaicism of a *DMD* truncating variant presenting with apparently isolated cardiomyopathy

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Background: Dystrophinopathies are a heterogeneous group of X-linked diseases, caused by pathogenic variants in the *DMD* gene, that mainly affect skeletal and cardiac muscle, but can also involve the brain. To date, there are nine reported cases of male affected patients with a pathogenic *DMD* variant's mosaicism. These patients presented with cardiac involvement and asymmetrical muscle weakness. A single case of isolated cardiac involvement, asymptomatic creatine kinase (CK) elevation and limited radiological signs of muscle wasting has been described.

Material and Methods: We here describe a male patient, who came to our attention at 30 years of age. Persistent troponin elevation and chest pain initially suggested myocarditis, however the cardiac MRI revealed heart hypokinesia, slight dilation and significant late enhancement with adipose replacement. Exome sequencing (ES) on a peripheral blood sample was requested due to suspicion of a cardiomyopathy.

Results: ES identified a mosaic pathogenic variant of the *DMD* gene: c.6283C>T, p.(Arg2095Ter), with an allelic fraction of 55%. Our patient did not present any symptoms related to skeletal muscle involvement and his CK levels were slightly above the normal range (204 mU/mL, n.v. 24–190 mU/mL). Neuropsychological evaluation was suggested, due to internalizing traits shown at clinical evaluation and excessive dependance upon his mother, despite his age.

Conclusion: Our case further expands the clinical spectrum associated with *DMD* mosaic variants in males, which can present with dilated cardiomyopathy and bizarre behaviors, hinting to an atypical neurodevelopment, without skeletal muscle involvement or severe hyperCKemia.

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P08 Metabolic and Mitochondrial Disorders

P08.001.D Survival, functional outcomes and safety in patients with thymidine kinase 2 deficiency (TK2d) and an age of TK2d symptom onset ≤12 years who received pyrimidine nucleos(t)ide therapy

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Background: Thymidine kinase 2 deficiency (TK2d) is an ultra-rare, autosomal recessive, mitochondrial myopathy. Early age at symptom onset is associated with rapid disease progression. Pyrimidine nucleoside therapy with doxecitine and doxribtimine is in development. We assessed survival, functional outcomes and safety in patients with age of TK2d symptom onset ≤12 years who received pyrimidine nucleos(t)ides.

Material and Methods: Treated patients were pooled from retrospective (NCT03701568, NCT05017818) and prospective (NCT03845712) sources and company-supported expanded access programs (EAPs; functional outcomes not collected). Untreated patients were pooled from literature reviews (2019; updated 2021) and a retrospective chart review (NCT05017818). Survival in 50th-percentile treated and untreated patient

matched-pairs was assessed using proportional hazard and marginal Cox models, and restricted mean survival time analyses. Functional outcomes (developmental motor milestones; ventilatory/feeding support) were compared pre- and post-treatment.

Results: Analyses included 82 treated (median [Q1, Q3] treatment duration: 54.8 [15.2, 78.4] months) and 93 untreated patients with age of symptom onset ≤12 years. Survival and functional outcomes are summarized in tables 1 and 2, respectively. In the safety population (age of symptom onset ≤12 years, n = 50; EAPs excluded), two patients (4.0%) discontinued treatment due to TEAEs; among those with data, diarrhoea was the most common TEAE (33/39 [84.6%]).

Conclusion: In patients with age of TK2d symptom onset ≤12 years, pyrimidine nucleos(t)ide therapy substantially improved survival and was well tolerated; stabilization or improvement in functional outcomes was observed.

Table 1. Survival outcomes for patients with age of TK2d symptom onset ≤12 years

	Untreated patients	Treated patients
Patients who died, n/N (%)	53/93 (57.0)	3/82 (3.7)
Proportional hazard and marginal Cox model estimates of reduced risk of death (compared with untreated patients)		
In the time from symptom onset	-	92–94% (hazard ratio = 0.06–0.08; $p < 0.0001$)
In the time from treatment initiation	-	87–95% (hazard ratio = 0.05–0.13; $p < 0.0001$)
Restricted mean survival time (95% confidence interval), years		
Over 30 years from symptom onset	14.4 (11.1–17.6)	29.2 (28.2–30.3)
Over 6 years from treatment initiation	2.8 (2.2–3.5)	5.8 (5.5–6.0)

Table 2. Functional outcomes in treated patients with age of TK2d symptom onset ≤12 years

		Pre-treatment	Post-treatment
Loss and regain of motor milestones			
Motor milestone loss	At risk, n [n1]	49	46
	Not at risk, n	33	36
	Lost ≥1 milestone, n/n1 (%)	41/49 (83.7)	10/46 (21.7)
	Lost ≥4 milestones, n/n1 (%)	20/49 (40.8)	1/46 (2.2)
Regain of previously lost motor milestones	At risk, n [n2]	41	40
	Not at risk, n	41	42
	Regained ≥1 milestone, n/n2 (%)	2/41 (4.9)	30/40 (75.0)
	Regained ≥4 milestones, n/n2 (%)	0/41 (0)	9/40 (22.5)
Use of ventilatory and feeding support during treatment			
Ventilatory support	Using support at treatment initiation, n [n3]	31	-
	Missing data, n	29	-
	Reduced hours of use post-treatment, n/n3 (%)	-	5/31 (16.1)
	Discontinued support post-treatment, n/n3 (%)	-	5/31 (16.1)
Feeding support	Using support at treatment initiation, n [n4]	19	-
	Missing data, n	30	-
	Discontinued support post-treatment, n/n4 (%)	-	2/19 (10.5)

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P08.002.A Integrative transcriptomics in primary mitochondrial myopathies: toward insights for diagnosis and stratification (Award Candidate)

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Background: Primary mitochondrial myopathies (PMM) are mitochondrial disorders mainly affecting skeletal muscle but with possible dysfunction of multiple organ systems resulting in highly variable clinical phenotypes and prognoses. PMM arises from genetic defects in mitochondrial DNA (mtDNA) including mtDNA point mutations and large-scale single deletions, or nuclear DNA (nDNA) genes causing multiple mtDNA deletions. This study investigates gene expression and transcriptomics pathways analysis in muscle biopsies from PMM patients to refine genotype-phenotype correlations and uncover molecular mechanisms and potential biomarkers for diagnosis and stratification.

Material and Methods: Muscle biopsies from a total of 100 PMM patients with large-scale mtDNA single deletions (MacroDel), multiple mtDNA deletions (MultiDel), or the m.3243A>G variant (m.3243) were analyzed versus disease-controls (muscles from subjects without any evidence of mtDNA alterations). RNA sequencing (RNA-seq) was performed to identify differentially expressed genes (DEGs) and pathway enrichment.

Results: Preliminary RNA-seq data analysis of the different subgroups with MacroDel, MultiDel, and m.3243A>G each versus controls revealed a significant number of DEGs and altered pathways (e.g. oxidative phosphorylation; cholesterol metabolism; translation; aerobic respiration; thermogenesis···). The study is currently ongoing to expand the analysis to the whole cohort.

Conclusion: Integrated analysis of RNA-seq data from muscle biopsies of well-defined cohorts of PMM patients with known mtDNA defects is expected to provide insights into the molecular mechanisms underlying these conditions. The integration of genetic, transcriptomic and clinical data can be useful to stratify patients, identify potential biomarkers, support personalised therapeutic approaches and contribute to the development of targeted therapeutic strategies.

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P08.003.B Accurate quantification of mitochondrial DNA heteroplasmy and copy number at the single cell level with digital PCR (Award Candidate)

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Background: Mitochondrial DNA (mtDNA) heteroplasmy and copy number are critical parameters influencing mitochondrial function and disease phenotypes. Current prevention strategies, such as preimplantation genetic testing (PGT) and nuclear transfer (NT), mainly rely on the analysis of single-cell biopsies. However, existing methods often lack the sensitivity for detecting small yet clinically relevant changes. Digital PCR (dPCR) offers a precise alternative with enhanced sensitivity, reproducibility, and reduced noise.

Materials and Methods: We developed a dPCR assay to quantify mtDNA copy number and heteroplasmy in single cells. Using a mouse model carrying the m.5024C>T mutation, we analysed unmanipulated mutant and pronuclear transfer (PNT) embryos across various stages, including oocytes, polar bodies, blastomeres, and trophectoderm (TE) and inner cell mass (ICM) of blastocysts. Validation was performed with synthetic templates of known mutation loads (0.01–50%). DPCR results were compared with next generation sequencing (NGS).

Results: dPCR reliably detected mutation loads as low as 0.1%. Single-cell analysis of cleavage stage embryos revealed no significant differences in heteroplasmy or mtDNA copy number between blastomeres. Strong correlations were found between oocytes and polar bodies; and between ICM and TE of blastocysts. At high mutation levels (50–80%), dPCR and NGS demonstrated strong concordance. However, in PNT embryos with low mutation loads (<4%), dPCR accurately quantified minor alleles (<1%), while NGS produced more false positives. In non-mutant samples, dPCR detected minimal mutant alleles (~0.07%), while NGS reported mutation loads of ~0.6%.

Conclusion: dPCR provides precise mtDNA quantification, outperforming NGS at low mutation levels with superior accuracy and fewer false positives.

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P08.004.C Re-evaluation of children with undiagnosed intrahepatic cholestasis identifies novel genetic causes

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Background: Familial intrahepatic cholestasis(FIC) comprise a heterogeneous group of metabolic liver diseases which may be progressive leading to liver failure. More than 15 monogenic causes have been identified as causative. Genes like *PPM1F, WDR83OS, LSR, VPS50, UNC45A, SLC51B* have been reported in only a few patients and disease association is not definite. Despite using broad spectrum genomic test like exome sequencing(ES), approximately 50% remain without a molecular confirmation.

Material and Methods: We selected 11 patients with a clinical diagnosis of FIC without a molecular confirmation despite ES. A reanalysis of the ES data was performed in all followed by repeat ES in 3 of them.

Results: All patients except one had features suggestive of progressive FIC. All had normal GGT levels. Liver biopsy was performed in 5 and showed features consistent with FIC. Reanalysis of prior focussed ES data showed poor coverage of many recently identified genes. Pathogenic variants were detected in *USP53* and *LSR* by repeat ES in 2 patients. Variants in probable novel candidate genes - *PATJ, EPHX2* were also identified. 2 patients with compound heterozygous variants in *PATJ* share a common variant and showed mild cholestasis. A patient with recurrent cholestasis without progressive liver disease was found to harbor a monoallelic pathogenic variant in *SEMA7A*.

Conclusion: Molecular diagnosis still remains elusive in a significant proportion of FIC cases. On re-evaluation we could confirm genetic etiology in 2 of 11 patients and add *PATJ* and *EPHX2* to the expanding list of probable candidate genes.

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P08.005.D Expanded newborn screening of phenylketonuria in Ukraine: genetic landscape

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Background: Phenylketonuria (PKU) is the inherited metabolic disease that causes the accumulation of phenylalanine and leads to intellectual disability. This disorder could be effectively diagnosed at an early stage within neonatal screening (NS). In Ukraine NS for PKU started in 2000 and confirmatory testing was presented by RFLP analysis of 9 pathogenic variants in the *PAH* gene. In October 2022, an expanded NS program was started followed by expanding genetic testing for diagnosis confirmation. It allowed the study of the *PAH* gene mutation spectrum in the Ukrainian population.

Material and Methods: DBS, LC-MS/MS, IFA, NGS, Sanger sequencing.

Results: From October 2022 to December 2024, 354 696 newborns from 4 regional screening centers were examined and 76 patients with PKU were confirmed. The incidence of PKU in Ukraine was determined to be 1:4 653 (excluding the occupied territories). A total of 45 distinct rearrangements were detected. In the study two novel variants were found - p.Thr186Lys and chr12:102854685-102877616del. The most common pathogenic variant was p.R408W (61/152, 40,1%). There were 11 patients with the p.R408W in both alleles. Other frequent pathogenic variants were p.A403V (16/152, 10,5%) and p.R158Q (7/152, 4,7%). Most of the rearrangements were found in exons 12 (55,9%), 7 (13,8%) and 11 (7,2%), therefore the analysis of these exons is proposed to be used as the first step of molecular genetic diagnosis of PKU in Ukraine.

Conclusion: Expanding of *PAH* molecular genetic testing allows studying the pathogenic variants spectrum in the Ukrainian population and improves the diagnosis algorithm of PKU.

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P08.006.A Background splicing of the *GALNS* gene unveils the hotspots for pathogenic splice-altering variants

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Background: For 10%–15% of patients with biochemical phenotype of Mucopolysaccharidosis Type IVA (MPS IVA), standard molecular-genetic testing fails to identify one or both causative variants in the GALNS gene. At least part of these variants is located deep in the GALNS introns and leads to pseudoexons (PEs) activation. Also, some of the reported exonic variants may alter GALNS splicing but are misclassified as, for example, missense variants. Information on the background or nonproductive splicing may indicate hotspots for such variants and serve as strong predictor of their outcome.

Material and Methods: The studied variants were collected after bioinformatic analysis of public repositories of the GALNS gene variants - ClinVar, Human Gene Mutation Database and gnomAD. The selected variants were subjected to in vitro splicing analysis using minigene assay in HEK293T cells. Information about background splicing (BS) was obtained with Snaptron analysis of SRA2 data.

Results: We identified eight PE-activating variants in the GALNS gene and demonstrated their clustering within wild-type PEs, which are present in mRNA as part of background splicing. Also, we demonstrate that "weak" canonical exons of the GALNS gene serve as hotspots for splicing variants that exert their deleterious effect by altering splicing regulatory elements, such as splicing enhancers and silencers.

Conclusion: We confirm that wild-type PEs and weak canonical exons of the GALNS gene are hotspots for splice-altering variants and these loci should be closely examined during molecular genetic diagnostics to improve detection of otherwise elusive variants and deepen the understanding of MPS IVA pathogenesis.

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P08.008.C Whole-genome DNA methylation profiling and identification of differentially methylated regions in obese children (Award Candidate)

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Background: Hereditary factors account for 40–70% of BMI variance, underscoring a strong genetic predisposition to obesity. However, the rapid rise in obesity prevalence cannot be fully explained by genetics alone. DNA methylation, an epigenetic mechanism, has been increasingly linked to obesity. Altered methylation patterns have been observed in obesity-associated genes, as well as genes involved in hypoxia and inflammation. We hypothesize that children with obesity exhibit distinct DNA methylation patterns compared to controls without obesity.

Material and Methods: Whole-genome sequencing using Oxford Nanopore technology was performed on whole blood of 10 children with obesity (BMI SDS > 2.0) without known monogenic variants linked to obesity alongside 10 age- and sex-matched controls without obesity. All participants were screened for copy number variations in severe early-onset obesity genes before methylation analysis. Basecalling and methylation calling were conducted with Dorado and Modkit. Differential analysis identified differentially methylated positions and regions, which were subsequently subjected to functional enrichment analysis.

Results: A total of 226,073 significantly differentially methylated positions ($p < 0.01$) were identified, including sites within the promoters of key obesity-related genes such as *POMC*, *ADCY3*, *LDLR*, *LEPR*, and *PCSK9*. Consolidation of DMPs revealed 548 differentially methylated regions. Some of these DMRs were associated with genes involved in pancreatic secretion, protein digestion, and abnormal eating behavior.

Conclusion: Obese children exhibit distinct DNA methylation patterns in genes linked to obesity and related processes. These findings highlight the role of epigenetic modifications in obesity pathogenesis and its comorbidities.

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P08.009.D De novo mutations in *XRN1* linked to a novel dominant form of lethal mitochondrial cardiomyopathy

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Background: XRN1 has been well characterised as a 5′-3′ exoribonuclease involved in mRNA decay within cytosolic stress granules. Here we report an association of *XRN1* with human disease in a cohort of infants with genetically undiagnosed mitochondrial cardiomyopathy.

Material and Methods: Through Matchmaker Exchange, we collated a cohort of individuals with similar heterozygous de novo variants in *XRN1* identified independently through research and clinical genome sequencing (GS). Functional studies were performed on available patient-derived tissues.

Results: Six individuals from five unrelated families presented similarly with lethal perinatal cardiomyopathy and failure to thrive following an acute viral infection. GS of each individual identified a de novo *XRN1* variant in the poorly conserved C-terminal domain, resulting in near-identical frameshift mutations and premature truncation of the protein's C-terminal tail. Despite having no known role relating to mitochondrial function in mammals, quantitative proteomics conducted on patient skeletal muscle revealed a consistent mitochondrial disease phenotype characterised by a combined abundance defect in oxidative phosphorylation (OXPHOS) complexes. These findings were supported by impaired OXPHOS assembly, assessed by Blue Native PAGE, histochemistry, and reduced respiratory chain enzymatic activity in muscle, heart and liver of affected individuals. RNA-sequencing of patient skeletal muscle additionally measured an increase in mitochondrial genome transcription, indicating a response to mitochondrial stress.

Conclusion: We propose *XRN1* as a candidate gene associated with a novel form of autosomal dominant lethal perinatal mitochondrial cardiomyopathy. Functional studies on patient-derived cells are continuing to provide insights into the precise disease mechanism.

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P08.010.A Novel molecular mechanisms and treatment option for *LIG3*-dependent Chronic Intestinal Pseudo-Obstruction

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Background: Chronic intestinal pseudo-obstruction (CIPO) is characterized by severe and recurrent sub-occlusive episodes mimicking a mechanical obstruction in the absence of any lumen-occluding lesion. We identified a novel recessive syndrome due to DNA ligase 3 (*LIG3*) mutations in patients with CIPO, leukoencephalopathy and neurogenic bladder. *LIG3* mutations affect mitochondrial DNA maintenance, leading to defective energy

production. We aimed at identifying altered molecular pathways and developing possible targeted treatments to revert/ameliorate the syndromic CIPO-related cellular energy impairment.

Material and Methods: Patients and control derived fibroblasts were used for RNA sequencing (RNAseq), immunofluorescence, western blot, live cell growth, ATP, and Ca^{2+} levels assays. Fibroblasts were exposed to L-Glutamine (L-Gln), which was also administered to *LIG3*-CIPO patients. Gastrointestinal (GI) symptoms were measured via Gastrointestinal Symptom Rating Scale.

Results: RNAseq revealed a profound dysregulation in mutant fibroblasts *vs*. controls, with overexpression of genes involved in extracellular matrix development and remodeling and mitochondrial functions. Histopathology of gut biopsies of *LIG3*-mutant patients showed collagen and elastic fiber accumulation. Mutant fibroblasts exhibited impaired mitochondrial bioenergetics and altered Ca^{2+} homeostasis. Supplementation with high concentrations of L-Gln (6 mM) restored growth and ATP levels in *LIG3*-mutant fibroblasts and improved significantly GI and extra-GI symptoms in *LIG3*-mutant patients after 8 months of treatment.

Conclusions: Mitochondrial dysfunction and symptom profile related to *LIG3* deficiency can be rescued by L-Gln supplementation. Our results provide a clinically relevant translational approach paving the way to effective, gene-based treatments for CIPO.

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P08.011.B Sengers Syndrome Caused by biallelic *TIMM29* Variants and RNAi Silencing in *Drosophila* Orthologue Recapitulates the Human Phenotype

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Background: Sengers-syndrome (S.S) is a genetic disorder characterized by congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy and lactic acidosis. All reported cases were genetically caused by biallelic mutations in the *AGK* gene. We herein report a pathogenic variant in TIMM29 gene, as a novel cause of S.S. Notably, AGK and Tim29 proteins are components of the TIM22 complex, which is responsible for importing carrier proteins into the inner mitochondrial membrane.

Material and Methods: Clinical data of 17 consanguineous patients featuring S.S was obtained. Linkage analysis, and sequencing were used to identify the disease-causing gene. Tissues derived from the study participants and a Drosophila melanogaster model were used to evaluate the effects of TIMM29 variant on S.S.

Results: The patients presented with a severe phenotype of S.S, markedly elevated serum creatine-phosphokinase, combined mitochondrial-respiratory-chain-complexes deficiency, reduced pyruvate-dehydrogenase complex activity, and reduced adenine nucleotide translocator 1 protein.Histopathological studies showed accumulation of abnormal mitochondria. Homozygosity mapping and gene sequencing revealed a biallelic variant in *TIMM29* NM_138358.4:c.514T>C NP_612367.1:p.(Trp172Arg). The knockdown of the *Drosophila TIMM29* orthologous gene (*CG14270*) recapitulated the phenotype and pathology observed in the studied cohort. We expand the clinical phenotype of S.S and provide substantial evidence supporting *TIMM29* as the second causal gene of a severe type of S.S, designated as S.S- *TIMM29*.

Conclusion: The present study uncovers several biochemical differences between the two S.S types, including the hyperCPKemia being almost unique for S.S-TIMM29 cohort, the different frequency of MMRCC and PDHc deficiencies among the two S.S types.

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P08.012.C Biallelic Variants in Alkaline Ceramidase 3 Cause Infantile and Early Childhood Onset Neurodegeneration with Progressive Leukodystrophy

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Background: Biallelic variants in Alkaline Ceramidase 3 (*ACER3*) have recently been linked to early-onset leukodystrophy in 3 case reports describing 5 families with 7 patents. We describe previously unreported 57 patients from 54 unrelated families with biallelic variants in ACER3 and cumulatively delineate the phenotypic spectrum of *ACER3*-related disease.

Material and Methods: Exome sequencing, extensive data sharing, and GeneMatcher were used to identify the patients reported here. ACER3 enzyme activity, lipidomics in patient fibroblasts, mutagenesis, functional assays, and protein modeling were performed.

Results: The cohort is composed of 64 patients from 59 families including 34 females and 30 males. 45 individuals are currently alive with a mean age of 6.6 ± 4.9 years (range 1.4–18). 19 patients (30%) died between the ages of 3 and 19 years due to the rapidly progressive disease. The disease presents with predominantly infantile-onset (86%), moderate (52%) and rapidly (39%) progressive neurological deficit manifesting with global developmental delay (71%) or developmental regression (98%)/stagnation (83%) commonly resulting in limbs spasticity (93%), limb dystonia (72%) and axial hypotonia (73%) with invariable posterior gradient white matter signal changes and diffuse cerebral volume loss (73%) on neuroimaging. Functional studies reveal significantly reduced ceramide hydrolysis. Mutant analysis identifies functional hot spots, with in vitro assays showing a 2–40% decrease in ceramidase activity. Lipidomic analysis confirms a 50% increase in ceramide and sphingomyelin levels, alongside reduced sphingosine.

Conclusion: Biallelic variants in ACER3 are associated with infantile and childhood-onset neurodegeneration with progressive leukodystrophy.

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R. Kaiyrzhanov: None. **R. Maroofian:** None.

P08.013.D SGLT1 functional polymorphisms are associated with early postprandial glycemic variability

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Background: The sodium/glucose co-transporter 1 (SGLT1) is encoded by *SLC5A1* and is primarily responsible for the intestinal uptake of glucose. Homozygous mutations of *SLC5A1* cause glucose/galactose malabsorption (GGM [MIM: 606824]). Subjects with normal glucose levels (normotolerant, NGT) at fasting and 2 hours after a glucose tolerance test (OGTT) but with 1h-OGTT ≥ 155 mg/dL (NGT-1h-high) have a worse cardio-metabolic risk profile compared to those with 1h-OGTT glucose < 155 mg/dl (NGT-1h-low). We investigated whether a haplotype with 3 missense mutations (p.N51S, p.A411T, and p.H615Q) of *SLC5A1* affected early postprandial glucose levels (1h-OGTT), and the functional significance of these variants.

Material and Methods: 800 metabolically well-characterized adult NGT volunteers were genotyped, subjected to a 75g-OGTT and stratified as NGT-1h-high or NGT-1h-low. Site specific mutagenesis was performed to introduce the three variants into a cellular model of human intestinal epithelium (CACO2).

Results: MAFs were higher ($P < 0.05$) in NGT-1h-low (1.515% N51S, 9.139% A411T, 3.926% H615Q) than in NGT-1h-high subjects (0.549%, 1.315%, 1.515%, respectively). Computation of a genetic risk score revealed that rare alleles conferred a dose-dependent lower risk of being classified as NGT-1h-high (OR 0.419, CI95% 0.217–0.810, $P < 0.01$). Glucose uptake was significantly reduced ($p < 0.0001$) in CACO2 expressing mutant SGLT1 (% reduction = ~29% N51S, ~30% A411T, ~33% H615Q). Cells carrying double or triple mutations showed even weaker glucose transport ability compared to wild-type (37–49%, $p < 0.0001$).

Conclusion: These data suggest that heterozygous *SLC5A1* missense variants may contribute to early post-prandial glycemic patterns, and that targeting intestinal glucose uptake might be a feasible strategy for preventing the formation of dysglycemia and long-term cardio-metabolic complications.

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P08.015.B Nanopore long-read NGS for the study of structural variants in the mitochondrial genome

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Background: Mitochondrial DNA (mtDNA) point mutations and large deletions or duplications are linked to neuromuscular and neurodegenerative diseases, cancer, and diabetes. While short-read next-generation sequencing (NGS) is the gold standard for mtDNA analysis, it is limited in detecting structural variants (i.e. large-scale deletions, duplications, and complex rearrangements). To overcome this limitation, we evaluated Nanopore long-read NGS.

Material and Methods: Whole DNA samples isolated from muscle biopsy of patients diagnosed for carrying single large (N = 30) and multiple mtDNA deletions (N = 30) were analyzed. DNA samples were processed according to Nanopore Rapid Sequencing Kit, a PCR free protocol, and sequenced on MinION/PromethION device. Reads were aligned to rCRS mtDNA sequence, using minimap2 and ngmlr; structural variants were called using Sniffle tool, combined with an in house pipeline.

Results: Nanopore long-read NGS analysis allowed us to easily detect single large deletion breakpoints and calculate deletion heteroplasmy. Long-read allowed to better characterize and quantify alterations of mtDNA molecules in samples affected by multiple deletions. Through an in house pipeline, we also identified long-reads with sequences compatible with complex mtDNA rearrangements, not detectable through the use of standard approaches or short-read NGS.

Conclusion: Since NGS has become the method of choice for genetic studies including the analysis of mtDNA, we think it is extremely important that geneticists and researchers are aware of the multiple NGS approaches available, including those based on long-reads, which can allow to overcome some limitations of standard short-read NGS.

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P08.016.C Genetic correction of metabolic diseases in patient cellsand mouse models

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Background: Inherited metabolic diseases can be treated by correcting the underlying genetic change in a target tissue. Many disease genes are active in the liver and editable with viruses, lipid nanoparticles or enveloped delivery vehicles. The argininosuccinatic aciduria (ASLD), methylmalonic acidemia (MMA), primary hyperoxalouria (HP1), and propionic acidduria (PA) are all potentially life-threatening recessive liver diseases which cause metabolic symptoms and can be envisioned to be treated with genetic correction.

Material and Methods: We created induced pluripotent cells (iPSCs) of patients with genetic metabolic diseases and CRISPR-Cas9 single nucleotide editing tools to target the specific genetic alterations, some of which represent founder mutations in the Finnish population. The corrections were made using different delivery vehicles, enabling the comparison of the efficacy, and the cellular phenotypes measured with biochemical methods and/or imaging. Lastly, we created a mouse model of the ASLD Finnish founder mutation and tested the different delivery strategies of in vivo correction.

Results: We describe the successful correction of four metabolic gene alterations with different genome editing tools and delivery systems in the patient iPSCs. We also show the improved cell phenotype as a result of the correction. The in vivo data shows high gene editing in the mouse liver tissue, and the phenotype analyses are ongoing.

Conclusion: Our results show that genetic alterations can be corrected in vitro in patient cells and in vivo in mouse liver. This

supports the development of disease correction therapeutic interventions in metabolic diseases of the liver.

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P08.017.D Transcriptomics on yeast models to deepen the knowledge of mitochondrial disease-associated pathways

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Background: Primary mitochondrial disorders and different forms of neurodegeneration can be associated with mitochondrial dysfunction. Most mitochondrial proteins are encoded by nuclear genes, imported into the organelle thanks to the mitochondrial targeting sequence, cleaved by specific peptidases (e.g. PITRM1) and released into the mitochondrial matrix. Biallelic *PITRM1* variants have been identified in patients with complex mitochondrial disorders (MD) whereas single heterozygous variants with Alzheimer disease (AD). Yeast models show an easy-detectable phenotype in mitochondrial defects, so we apply a hypothesis-free approach based on whole transcriptomics to identify new impaired cellular or mitochondrial pathways to improve our knowledge on the pathomechanisms.

Material and Methods: We focused our studies on CYM1 and STE23, encoding mitochondrial peptidases (respectively PITRM1 and IDE human orthologues). We performed RNAseq on single and double knock-out yeast models and on strains harbouring the corresponding MD or AD human PITRM1 variants. Transcriptomics data were used to identify differentially expressed genes (by DESeq2) and to detect dysregulated pathways (using GSEA package). Mitochondrial metabolism assays, functional and proteomics analyses were also performed.

Results: The most up-regulated genes in the mutant strains were implicated in the regulation of proteolysis, while in the KO-strain in the metabolism of NADP. In AD-group, down-regulated genes were involved in membrane biosynthetic processes, while in MD-group in helicase activity. Some of these findings correlated with functional and proteomic data.

Conclusion: Transcriptomics on yeast models can increase knowledge about the molecular pathogenesis in mitochondrial diseases and identify impaired pathways, which can represent novel therapeutic targets.

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P08.018.A Exploring heteroplasmic variants in mtDNA: insights from single-cell transcriptomics

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Background: The study of mitochondrial heteroplasmy, defined by the coexistence of wild-type and mutant variants of mitochondrial DNA (mtDNA), is crucial for understanding biological processes and pathologies associated with mtDNA. Bulk RNA sequencing has long been established as the primary method for profiling mtDNA mutations and assessing their effect on trascriptomics; however, this approach doesn't account for the considerable variability in heteroplasmy among individual cells, where thousands of different mtDNA molecules exist. Our aim was to study the distribution of mtDNA variants in scRNA-seq data and their link with global gene expression.

Material and Methods: Four fibroblast cell lines from patients with different heteroplasmic mtDNA variants (m.11777C>A, m.13513G>A, m.13514A>G, m.13063G>A) were analyzed. Based on a 10x Genomics protocol, a data analysis workflow was implemented utilizing integrated bioinformatics pipelines: Cell Ranger, Mitotrace, Seurat, and DEsingle within Linux-based and R environments. Input data included BAM files, rds files containing read count matrices, and reference genomic sequences in GRCh38.fasta format.

Results: The mean heteroplasmy at RNA level was comparable to that identified in DNA. All samples showed a bimodal distribution with most of the cells close to homoplasmic wild-type or homoplasmic mutant status. The comparison of these two extreme subpopulations showed some differences in gene expression, used for pathways enrichment analysis.

Conclusion: We evaluated a novel approach for identifying the distribution of mtDNA variants in scRNA-seq data. This has allowed a detailed examination of how mutant heteroplasmy status influences gene expression differences, giving exciting prospects for investigating mtDNA heteroplasmy across various clinical contexts.

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M. Barresi: None. **I. Di Meo:** None. **A. Nasca:** None. **E. Lamantea:** None. **A. Legati:** None. **D. Ghezzi:** None.

P08.019.B Insights into the pathophysiology of *POLG2* mutations leading to early onset mtDNA deletion and depletion syndrome

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Background: Several mitochondrial disorders are characterized by depletion of mitochondrial DNA (mtDNA) and accumulation of multiple mtDNA deletions due to mutations in genes involved in mtDNA maintenance (mtDNA maintenance defects -MDMDs). Mutations in POLγ, the mtDNA replicative polymerase, represent the major cause of mtDNA instability resulting into MDMDs. POLγ is a trimeric complex constituted by one major replicative subunit, POLG1, and two smaller accessory subunits, POLG2. One of the proposed roles of POLG2 is the preservation of the integrity of the POLγ holoenzyme. We recently identified a homozygous mutation in the POLG2 gene in a patient affected by early onset acute liver failure.

Material and Methods: We established a primary fibroblast culture from the patient's skin biopsy and characterized its biochemical and molecular profile using Seahorse analysis, real-

time PCR and western blot analysis. In parallel, we are generating a zebrafish knock-in (KI) polg2 mutant line harboring the patient's mutation to study its effect in vivo.

Results: Our preliminary results showed that the analysed mutation impairs mitochondrial respiration and cause mtDNA depletion and a change in the levels of different mitochondrial proteins involved in mitochondrial replication.

Conclusion: Our data show the impact of mutation on POLG2 gene on the process of mtDNA maintenance and replication. The developed models will be instrumental to test the effect of specific molecules targeting the stability of POLγ holoenzyme.

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P08.023.B Severe neonatal hypotonia caused by *SLC30A5* variant affecting function of ZnT5 zinc transporter

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Background: The tightly-regulated spatial and temporal distribution of zinc ion concentrations within cellular compartments is controlled by two groups of Zn^{2+} transporters: the 14-member ZIP/SLC39 family, facilitating Zn^{2+} influx into the cytoplasm from the extracellular space or intracellular organelles; and the 10-member ZnT/SLC30 family, mobilizing Zn^{2+} in the opposite direction. Genetic aberrations in most zinc transporters cause human syndromes. Previous studies demonstrated osteopenia and male-specific cardiac death in mice lacking the ZnT5/*SLC30A5* zinc transporter, and suggested association of two homozygous frameshift *SLC30A5* variants with perinatal mortality in humans, due to hydrops fetalis and hypertrophic cardiomyopathy.

Material, Methods and Results: We studied the molecular basis of a novel severe hypotonia syndrome. Homozygosity mapping combined with exome sequencing studies of consanguineous Bedouin kindred, as well as transfection experiments and zinc monitoring in HEK293 cells, demonstrated that a bi-allelic in-frame 3 bp deletion variant in *SLC30A5*, deleting isoleucine within the highly conserved cation efflux domain of the encoded ZnT5, results in lower cytosolic zinc concentrations, causing a syndrome of severe non-progressive neonatal axial and limb hypotonia with high-arched palate and respiratory failure. There was no evidence of hydrops fetalis, cardiomyopathy or multi-organ involvement. Affected infants required nasogastric tube or gastrostomy feeding, suffered from various degrees of respiratory compromise and failure to thrive, and died in infancy.

Conclusion: A biallelic SLC30A5 (ZnT5) variant, affecting cytosolic zinc concentrations, causes a severe hypotonia syndrome with respiratory insufficiency and failure to thrive, lethal by 1 year of age.

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P08.024.C Expanding the genetic and clinical spectrum of LBSL: characterization of a Russian cohort of 206 patients

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Background: Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is a rare inherited disorder caused by biallelic pathogenic variants in the DARS2 gene. The vast majority of patients harbor a common variant c.228-21_228-20delinsC in a compound heterozygous state, which reduces exome sequencing yield and obscures genotype-phenotype correlations for LBSL. Several large deletions have also been reported, further complicating the disease investigation.

Material and Methods: The diagnosis was confirmed by genealogical analysis, clinical presentation, brain MRI, and Sanger sequencing of DARS2 exons. Whole-genome sequencing was performed for one patient.

Results: We investigated 206 patients (aged 6 months-53 years) diagnosed over 15 years. Three forms were identified based on age of onset: early (0–4 years, 49%), childhood (5–14 years, 35%), and late (≥15 years, 16%). The most common symptoms were cerebellar ataxia and axonal neuropathy. The first symptoms included gait disturbances, leg weakness, and, rarely, seizures or tremors. In most cases (77% of 127 patients), the disease manifested in asymptomatic individuals, while in 10% cases it was associated with a traumatic brain injury and in 10% with viral infections, highlighting their potential role as triggers. Genetic analysis identified 27 unique DARS2 variants, including 13 novel. A large deletion spanning exons 16–17 in four patients, may represent a distinctive feature of the Russian population. Additionally, we describe a unique case of two patients with the common variant c.228-21_228-20delinsC in the homozygous state.

Conclusion: Our findings broaden the LBSL clinical and genetic landscape by reporting new pathogenic variants.

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P08.025.D Spatial transcriptomics reveals altered *TMSB4X* expression in Fabry disease

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Background: Fabry disease is the most common lysosomal storage disorder, characterized by hypertrophic cardiomyopathy and subclinical chronic inflammation. In a subset of patients, significant organ damages accompanied by systemic inflammation can be observed. We hypothesized that the exacerbation of the chronic inflammatory condition may significantly contribute to the development of acute organ dysfunction. Our aim was to immunologically characterise the explanted heart of a Fabry disease patient with cardiomyopathy undergoing heart transplantation, and circulating T lymphocytes of Fabry patients using spatial and single cell transcriptomics.

Material and Methods: 10x-based spatial transcriptomics of explanted heart and further single cells transcriptomics analysis of negatively FACS sorted circulating T lymphocytes of Fabry patients (N = 7) and age-matched controls (N = 8) was performed using the 5' gene expression kit (10 000 cells/patient). Quality control, clustering and gene expression analysis was performed in Scanpy v.1.10.

Results: Spatial transcriptomics reveals an abundant NPPA expression within the inner endothelial lining could be observed. Within striking distance cardiac resident CD163+ macrophages in explanted heart tissue show a higher TMSB4X expression compared to cardiomyocytes or cardiac patrolling monocytes. We have also identified a modified TMSB4X expression pattern in circulating T cells of Fabry patients.

Conclusion: Our preliminary data suggests that NPPA together with TMSB4X may play a significant role in overturning equipoise towards organ dysfunction.

C.O.I. Disclosure:

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P08.026.A Expansion of the clinical and molecular spectrum of sphingomyelin synthase 1 deficiency

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Background: Several genes involved in sphingolipid metabolism have been implicated in lipid storage disorders. Sphingolipids are critical for the composition of cell membranes, in particular facilitating vesicle trafficking. *SGMS1*, encoding sphingomyelin synthase 1 (SMS1), which catalyzes the reaction from ceramide to sphingomyelin, has not yet been linked to a Mendelian disease, except for our oral presentation at last year's ESHG conference.

Material and Methods: Using CRISPR-Cas9 and base editing, three different *SGMS1* missense variants were introduced into HeLa cells. Furthermore, we generated *SGMS1* knockout cells. We subsequently investigated the phenotype of the cell lines using quantitative RT-PCR, immunofluorescence and lipidomics.

Results: We present the clinical data of now five individuals from three unrelated families harboring biallelic *SGMS1* missense variants. All affected individuals presented with a neurodevelopmental delay phenotype. The three missense variants were confirmed to result in SMS1 deficiency. Additionally, abnormal morphology of the Golgi apparatus was detected in immunofluorescence staining for all variants. The most aberrant morphology was observed in the *SGMS1* knockout cell line indicating disrupted intracellular trafficking processes as a result of the loss-of-function of SMS1 protein.

Conclusion: We further delineate the clinical and molecular spectrum of affected individuals with SMS1 enzyme deficiency. Our data strengthen the assumption that biallelic SGMS1 variants are causative for a so far unpublished hereditary metabolic disorder.

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P08.027.B Alteration of 3-D chromatin structure as a novel mechanism involved in inherited metabolic diseases

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Background: The application of different omics technologies beyond the exome can solve the most elusive cases with inherited metabolic diseases (IMD), reducing the diagnostic gap.

Material and Methods: Transcriptomic studies were performed in combination with targeted long-read sequencing (T-LRS) using nanopore technology (MinION sequencer), as well as Circular Chromosome Conformation Capture (4C) studies to determine the cause of two patients' pathology. These studies were performed in DNA and RNA extracted from patients' fibroblasts.

Results: We have selected two patients with clinical and biochemical diagnosis of two different IMD: maple syrup urine disease and GLUT1 deficiency syndrome. The transcriptomic analysis revealed reduced mRNA levels of *DBT* and *SLC2A1*, respectively. The T-LRS results allowed us to identify a hypermethylation defect in *DBT* promoter and the insertion of a transposable element (LINE_L1) of 2.5 Kb in the 3' UTR region of *SLC2A1*. Methylation analysis (850k arrays) showed a specific methylation defect in the *DBT* promoter, as well as a pathology-specific epigenetic signature. No genomic variants have been detected in this case yet. 4C analysis, performed using the promoter of each gene as a viewpoint, revealed a loss of interactions between *DBT* and transcriptionally active genes in the first patient. In the second case, a loss of interactions between the *SLC2A1* promoter and its enhancer was observed, likely caused by the LINE insertion.

Conclusion: These findings broaden the mutational spectrum to include new non-standard variants, highlighting the importance of using different technologies to resolve all cases and defining the molecular and epigenetic mechanisms underlying IMD.

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P08.028.C Correlation of heteroplasmy variation between mother and child and maternal age at conception in two mitochondrial diseases

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Background: MtDNA is present in many copies in cells and different variants may coexist leading to the condition of "heteroplasmy" or "polyplasmy". Deleterious mtDNA variants need to cross a threshold of heteroplasmic load before developing clinical symptoms. During oogenesis, mtDNA undergoes a reduction of copy number, named "genetic bottleneck", that can shift drastically the heteroplasmic loads of mtDNA variants from mother to child. It is still investigated how selection against certain mtDNA variants may occur, purifying from pathogenic variants the pool of mtDNAs along the germline. In this study, we analyzed the relationship between the age at conception and the change in heteroplasmy from mother to child in two mitochondrial diseases.

Materials and methods: We considered 117 heteroplasmic carriers of the variants m.3243A>G or m.3460G>A responsible for MELAS and LHON syndromes, respectively. Heteroplasmy degree was determined in blood cells with the SNaPshot approach. We correlated the difference of heteroplasmy between mother and child and the maternal age at conception.

Results: We observed a consistent trend towards the reduction of mutant mtDNA in child compared to mother's heteroplasmy as maternal age at conception increased, for both m.3243A>G and m.3460G>A variants. This selection was more evident after 30 years of maternal age at conception, whereas it was not apparently influenced by maternal heteroplasmy.

Conclusions: This study suggests that there is a correlation between variation of heteroplasmy between mother and child and maternal age at conception. At our knowledge, this is the first evidence in human of a phenomenon recently described in a mouse model.

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P08.029.D Investigation of secondary lipids associated with inflammation in Neu1 deficient mice

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Background: Lysosomal Neuraminidase 1 (Neu1) is responsible for sialic acid removal from glycoproteins and sialoglycoconjugates. Neu1 mRNA was highly expressed in humans' pancreas, kidney, and liver but low in brain. Mutations in *NEU1* cause a rare lysosomal storage disorder, sialidosis. In previous studies, marked increase was shown in of GM3, GD3, GM4, and LM1 ganglioside levels in spleen, liver, and kidney of sialidosis patients. In mice, Neu1 expression is shown in highest levels in kidney and pancreas tissues, and less expression in brain, liver, and spleen. *Neu1-/-* mice model mimics Type II (severe form) of sialidosis with visceromegaly and severe kidney defects caused by vacuolization in glomeruli regions. In this study, we aimed to explore altered secondary lipid metabolism associated with inflammation in mice models of sialidosis.

Material and Methods: Shotgun lipidome and RT-PCR analysis were used to determine the levels of secondary lipids and inflammation-related gene expression in kidneys of 2- and 5-month-old *Neu1-/-* mice.

Results: We detected a remarkable decrease in secondary lipids of PA, PC, PE, and PI phospholipids, sphingomyelin, and cholesterol esters in both age groups. Besides our data showed significantly increased levels of pro-inflammatory cytokines, including Ccl2, Ccl3, Ccl4, and Ccl5, and anti-inflammatory cytokines Ccl1, Ccl7, Ccl19, Il-11, Il-4, Il-10, Il23a, and Il-12 in the 2- and 5-month-old kidneys of *Neu1-/-* mouse models.

Conclusion: Our results indicate that secondary lipid alteration is associated with inflammation in *Neu1-/-* mice, offering a novel, comprehensive insight into pathophysiological aspects that may guide preclinical researchers in future research.

C.O.I. Disclosure:

E. Ada: None. **V. Seyrantepe:** None.

P08.030.A Mutational spectrum of hypophosphatasia in Russian patients and investigation of ALPL gene splicing

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Background: Hypophosphatasia (HPP) is a rare inherited disorder associated with pathogenic variants in the *ALPL* gene, leading to reduced activity of tissue-nonspecific alkaline phosphatase (TNSALP). Main symptoms include impaired mineralization of bones and teeth, respiratory issues, seizures, muscle weakness, and bone pain. According to public mutation databases, pathogenic splicing variants in *ALPL* comprise about 6% which is lower than commonly expected (15%), indicating the need for deeper investigation of *ALPL* splicing.

Material and Methods: We present molecular genetic data of 82 patients with confirmed HPP diagnosis referred to our Centre during 2014–2024. Genotyping was performed using Sanger sequencing. To assess the impact of variants on splicing, we used minigene assay and HEK293T cells.

Results: In our patient's cohort, we identified 58 variants: missense (44, 76%), nonsense (1, 2%), synonymous (1, 2%), frameshift (4, 7%), in-frame deletions (3, 5%), in-frame insertion (1, 2%), and splicing variants (4, 7%). This distribution correlates well with data from the global *ALPL* mutation database (alplmutationdatabase.jku.at). To improve the classification of *ALPL* variants, using bioinformatical approaches we analyzed patient's variants and additional variants from public mutation databases and selected 228 that were predicted to affect splicing. The minigene system comprising all exons of the *ALPL* gene, demonstrated good recognition of exons and was validated with previously characterized splicing variants.

Conclusion: The *ALPL* variant spectrum in Russian patients correlates with global data. The developed minigene system is a reliable tool for functional analysis of splicing-affecting variants, advancing understanding of *ALPL* splicing in HPP.

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P08.031.B Genetic and Dietary Influences on Total Cholesterol Levels: APOE Gene-Diet Interactions in a Korean Population

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Background: Dyslipidemia, a prevalent metabolic disorder affecting approximately 40% of the Korean population, is influenced by both genetic predisposition and dietary intake. This study focused on the apolipoprotein E (APOE) gene to examine its role in modulating total cholesterol levels under varying dietary conditions.

Material and Methods: We conducted a genome-wide association study (GWAS) using 2,426 individuals from the Korean Genome and Epidemiology Study (KoGES), excluding participants with missing or extreme values. Two significant single nucleotide polymorphisms (SNPs) in APOE, rs429358 ($P = 0.0058$) and rs7412 ($P = 5.9 \times 10^{-9}$), were associated with total cholesterol levels. To investigate gene-diet interactions, we analyzed caloric intake, minerals, and vitamins.

Results: Among six minerals and eight vitamins evaluated, most minerals demonstrated consistent effects on cholesterol. An "inverted U relationship" emerged for carbohydrates, proteins, and fats, prompting their exclusion from further interaction analyses. The most notable modification was observed for vitamin B1 in individuals carrying rs7412 ($\beta = -17.052$). Additionally, calcium ($\beta = -4.68285$, $P = 0.02$) and vitamin A1 (retinol) ($\beta = -4.04966$, $P = 0.03$) showed significant interactions with rs7412, suggesting that increased intake of these nutrients may lower total cholesterol among carriers of this APOE variant.

Conclusion: These findings emphasize the importance of APOE-diet interactions in cholesterol regulation and suggest potential benefits from personalized nutritional interventions. Further studies with larger, controlled dietary cohorts are warranted to confirm these associations, and future research should also address potential ethnic differences in gene-diet interactions.

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M. Woo: None. **S. Lee:** None. **Y. Kim:** None. **S. Jeong:** None.

P08.032.C Unravelling phenylalanine-induced neuronal dysfunction: transcriptome analysis of NT2-derived neurons highlights neurite impairment and synaptic connectivity

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Background: Phenylketonuria (PKU) is the most common inherited disorder of amino acid metabolism, characterized by elevated phenylalanine (Phe) levels in the blood and brain, leading to cognitive impairment if untreated. However, Phe-mediated brain dysfunction is not fully understood. Here, we investigated gene expression alterations due to excessive Phe exposure in human neuronal model, providing additional molecular insights into PKU pathophysiology.

Material and Methods: NT2/D1 cells were differentiated in culture using retinoic acid for four weeks. Resulting NT2-derived neurons (NT2/N) were treated with varying concentrations of Phe (1.25, 2.5, 5, 10 and 30 mM) for seven days and subjected to whole-mRNA short-read sequencing. Differentially expressed genes (DEGs) were identified, followed by overrepresentation and enrichment analysis. To validate differential expression, qRT-PCR was performed.

Results: After treatment with three different Phe concentrations (2.5 mM, 5 mM, and 10 mM), DEGs highlighted *CDC42BPG*, *CDH6*, *GPR50, LRP4*, *PREX1*, *PCSK9*, *RASGRF2* (up-regulated) and *PRMT8* (down-regulated) genes, placing them in the context of PKU for the first time. qRT-PCR confirmed these findings. Overrepresented and enriched processes included neuron projection guidance, dendrite and axon impairment, synaptic transmission, and neurotransmitter uptake. In contrast, the 30 mM Phe treatment group clearly represented the neurotoxicity of Phe, exhibiting enrichment in apoptotic pathways, while 1.25 mM Phe treatment did not yield any DEGs.

Conclusion: We established NT2/N as a novel model for Phe-mediated neuronal dysfunction. Differential gene expression profiling and enrichment analysis revealed that exposure to applied Phe concentrations leads to neurite impairment and affects synaptic connectivity.

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P08.033.D Molecular genetic analysis of patients with Fabry disease and their prevalence in Ukraine

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Background: Fabry disease is an X-linked lysosomal storage disorder caused by a *GLA*-related alpha-galactosidase A deficiency. The peculiarity of the GLA gene is its extremely high heterogeneity, which causes difficulties in interpreting the results of molecular genetic analysis of individuals with suspected Fabry disease, especially females.

Material and Methods: blood samples of patients with suspected Fabry disease were examined by NGS and Sanger sequencing.

Results: The variants in the *GLA* gene were found in 41 patients (29 families) with suspected Fabry disease, including 29 men and 12 women. All women were identified through cascade family analysis of the primary male patient. Therefore, the calculation of quantitative indicators of the frequency of *GLA* gene variants was performed exclusively for the first male patients identified in each family. In total, 20 variants of the *GLA* gene were detected - missense (14/20; 70%), nonsense (3/20; 15%), and point deletions (3/20; 15%). A little more than half of the variants were pathogenic (11/20), others were probably pathogenic except two (p.Asp313Tyr and p.Ser126Gly) had an uncertain value or contradictory pathogenicity classifications. The most frequent variants were p.Arg112Cys (4/29; 14%) and p.Asp313Tyr (3/29;10%), and the remaining variants were unique i.e. met in one or two families at most.

Conclusion: The study results are the basis for further study of the genetic heterogeneity of the Fabry disease and contribute to the optimization of early diagnosis and genetic counseling in Ukraine.

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P08.034.A Mutational analysis of the *GCDH* gene in patients with glutaric aciduria type I in Ukraine

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Background: Glutaric aciduria type I (GA1) is a hereditary disease caused by *GCDH*-related deficiency of the glutaryl-CoA dehydrogenase. The spectrum and frequency of pathological variants in the *GCDH* gene vary depending on the population, which is taken into account when developing the algorithm for effective diagnosis of GA1.

Material and Methods: Bio samples of patients with GA1 were analyzed by biochemical (LC/MSMS, GC/MS), and molecular genetic (NGS, Sanger sequencing) methods.

Results: Since 2014, 13 patients (5 of them by NBS) with GAI were identified in Ukraine by metabolic markers (elevated C5DC, glutaric, and 3-OH glutaric acids) and *GCDH* gene analysis. 12 pathogenic variants were identified: 9 missense, one deletion in the coding region, and two splicing variants. Most of the rearrangements were localized in exon 11 of the *GCDH* gene (16/26, 61.5%). The p.Arg402Trp variant has the highest frequency (12/26, 46%). This variant has been described as the most common among Caucasians in various populations, from 20% in Turkey to 52% in Poland (Pokora et al, 2019). All other variants were rare, i.e., they were found in one, two, or three families.

Conclusion: The most common variant in the *GCDH* gene among patients with GA1 in Ukraine is the p.Arg402Trp, which is similar to the European populations. The localization of 61.5% of the detected variants in exon 11 makes it advisable to analyze this exon first in patients with the GA1 metabolic profile. This approach will speed up and reduce the cost of GA1 diagnostics.

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P08.035.B Beyond-Standard WES Data Analysis Reveals Mitochondrial Instability in a Patient with Chronic Muscle Pain and Fatigue

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Background: Mitochondrial DNA (mtDNA) is crucial for ATP synthesis, and its instability can lead to various diseases. Factors contributing to mtDNA instability include defects in replication, repair mechanisms, and accumulated mutations. Additionally, mitochondrial dysfunction has been linked to nuclear genome instability, suggesting a bidirectional relationship. Understanding mtDNA instability is essential for studying its role in disease and aging

Material and Methods: We performed exome sequencing and mtDNA sequencing in a young patient with chronic muscle pain and fatigue. Our analysis included detection and quantification of mtDNA rearrangements from paired-end sequencing data.

Results: Standard analyses did not reveal pathogenic SNVs or CNVs in nuclear DNA or mtDNA. While high-throughput sequencing has improved mtDNA variant detection, identifying mtDNA rearrangements, particularly multiple deletions, remains challenging. To address this, we applied **eKLIPse**, a specialized tool for detecting and quantifying mtDNA rearrangements. eKLIPse identified several low-level mtDNA deletions. Further analysis of DNA from uroepithelial and hair follicle cells confirmed mtDNA rearrangements in uroepithelial cells but not in hair follicle cells. The analysis of samples from mother was normal

Conclusion: Comprehensive WES-based mtDNA analysis revealed tissue-restricted mitochondrial instability, likely contributing to the patient's chronic muscle pain and fatigue. Further research is needed to elucidate the underlying mechanisms and clinical significance of this instability.

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P08.036.C Unraveling the spectrum of *RFX6* mutations associated with diabetes

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Background: Regulatory Factor X6 (RFX6) is a transcription factor essential for β-cell development and function. Biallelic *RFX6* mutations cause Mitchell-Riley syndrome, characterized by neonatal diabetes, pancreatic hypoplasia, and intestinal atresia. Heterozygous mutations are associated with diabetes onset in childhood or adulthood. This study examined the effects of a novel *RFX6* mutation (identified through exome sequencing in a family with autosomal dominant diabetes) and five previously reported mutations.

Material and Methods: Wild-type and mutant RFX6 proteins were expressed in HEK293 cells and analyzed for protein levels, nuclear localization, and transcriptional activity on the insulin promoter using Western blotting, immunofluorescence, and luciferase assays. Proteasomal degradation was assessed using the MG132 inhibitor.

Results: The novel p.Ile878Asnfs3 mutation significantly increased protein levels, abolished nuclear translocation, reduced transcriptional activity by 67%, and conferred resistance to proteasomal degradation. p.Arg181Gln significantly reduced nuclear localization and transcriptional activity by 55% and 67%, respectively. Truncating mutations (p.Leu292Ter and p.Lys351Ter) significantly reduced protein levels, nuclear localization (by 20% and 47%), and transcriptional activity (by 70% and 66%). p.Arg726Ter significantly reduced nuclear localization (by 12%), while p.Arg866Ter maintained high nuclear localization. p.Ile878Asnfs3 exhibited 100% penetrance, whereas p.Arg181Gln, p.Leu292Ter, and p.Lys351Ter had reduced penetrance (57%, 91%, and 50%, respectively) but remained a risk for diabetes in younger or prediabetic carriers. Notably, individuals with combined p.Arg726Ter/p.Arg866Ter developed Mitchell-Riley syndrome, despite neither mutation alone significantly impairing transcriptional activity.

Conclusion: These findings provide insights into RFX6-related diabetes mechanisms and variable penetrance, emphasizing the impact of specific mutations on disease presentation.

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P08.037.D Elevated soluble ACE2 in Gaucher patients : An evolutionary advantage?

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Background: Gaucher disease (GD) has a high carrier rate among Ashkenazi Jews, although the most common disease-causing variant, N370S, is pan-ethnic. This has led to speculations of evolutionary advantage for carriers, particularly of this variant. During the recent COVID-19 pandemic, GD patients reportedly had a surprisingly low infection rate and mild symptoms considering their disease status. As SARS-CoV-2 gains entry into the cell via membrane-bound angiotensin-converting enzyme 2 (ACE2), we speculated that differences in levels of soluble ACE2 in GD patients could contribute to this protective state. While ACE is a biomarker of GD, ACE2 levels have not been explored in GD.

Material and Methods: We measured serum levels of ACE and ACE2 by ELISA in 33 GD patients and 17 age- and sex-matched controls as well as macrophage bound ACE2 by western blot, in 7 GD and 7 age- and sex-matched controls.

Results: Our results revealed a significant elevation of both serum and macrophage-bound ACE and ACE2 in GD patients compared to healthy controls. Moreover, the most robust ACE2 elevation was observed in N370S homozygotes (60 ng/ml in homozygotes versus 20 ng/ml in controls; $P \leq 0.001$.), regardless of treatment status.

Conclusion: We provide preliminary evidence for significant soluble ACE2 elevation in GD patients, particularly those with the N370S homozygous genotype. Since coronaviruses use the ACE2 receptor as a gateway for host cell entry, we speculate that elevated circulating ACE2 may serve as a decoy, giving advantage to GD patients during viral infections, of which SARS-Cov-2 serves as a contemporary example.

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P08.038.A Mutational spectrum of X-ALD in Russian patients and investigation of the *ABCD1* gene splicing

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Background: X-linked adrenoleukodystrophy (X-ALD) is a recessive inherited neurodegenerative disease associated with biallelic variants in the *ABCD1* gene and leading to progressive demyelination of the nervous system and adrenal insufficiency. Although it is generally accepted that about 15% of all pathogenic variants alter splicing, the *ABCD1* splicing remains poorly understood.

Material and Methods: We present molecular genetic data of 198 patients with X-ALD who were referred to the Research Centre for Medical Genetics from 2004 to 2024 years. The diagnosis was confirmed by clinical and radiological examination followed by NGS and Sanger sequencing of *ABCD1*. HEK293T cells and pSpl3_Flu2_miniTK minigene vector were used to assay the impact of variants on splicing.

Results: In our patient's cohort, 140 unique variants were identified, including: indel variants - 17%, frameshift - 11%, nonsense - 23%, missense - 42%, splicing - 7%, which correlates well with literature data (https://adrenoleukodystrophy.info/). After bioinformatic analysis of patients' variants, we identified additional 17 that were predicted to affect splicing. We created a minigene system for all exons of the *ABCD1* gene and validated it on well-known *ABCD1* splicing variants. Further, to improve variant classification in public databases of X-ALD mutations we analyzed the Human Gene Mutation Database, ClinVar, and ALD.info database and selected another 102 variants that were predicted to affect splicing.

Conclusion: We demonstrated that our minigene system for *ABCD1* is suitable for functional analysis of variants that are predicted to affect splicing, which is what we are currently working on.

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P08.039.B Pancreatic magnetic resonance imaging in carriers of m.3243A>G mitochondrial disease with and without diabetes

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Background: The mitochondrial DNA variant m.3243A>G disrupts oxidative phosphorylation reducing mitochondrial adenosine triphosphate production, and is strongly associated with an elevated risk of diabetes mellitus (1). This pathophysiology likely arises from impaired beta-cell function and decreased insulin sensitivity (2); however, the precise mechanisms remain unclear. This study aimed to determine whether m.3243A>G carriers exhibit pancreatic morphological changes.

Material and Methods: Healthy controls, non-diabetic and diabetic m.3243A>G carriers were evaluated using a unique approach of magnetic resonance imaging (MRI) parameters of the pancreas. We measured gland volume to evaluate atrophy, T1 relaxation time and magnetic resonance elastography (MRE) stiffness for evaluation of fibrosis, and proton density fat-fraction (PDFF) for fat content (3).

Results: The study included 11 non-diabetic and 12 diabetic m.3243A>G carriers and 23 controls. T1 relaxation time ($p < 0.001$) and MRE-derived stiffness ($p = 0.03$) differed between groups. The diabetic carriers had a slightly higher median (interquartile range) T1 relaxation time [909ms (868–1085) vs. 823ms (785–852), $p = <0.001$] and MRE-derived stiffness [1.32kPa (1.24–1.43) vs. 1.23kPa (1.12–1.28), $p = 0.02$] than healthy controls, but non-diabetic carriers showed no differences from controls in these parameters. Pancreatic volume and PDFF did not differ among groups.

	Controls	m.3243A>G	Any m.3243A>G subdivided		p-value[a]
			Non-diabetic	Diabetic	
Number, *n*	23	23	11	12	-
Basic demographics					
Sex (Female/Male)	16/7	16/7	6/5	10/2	-
Age (years)	42.0 ± 14.2	41.7 ± 13.2	36.0 ± 13.0	46.8 ± 11.5	0.16 (overall)
Body mass index (kg/m^2)	23.9 ± 3.7	23.4 ± 5.1	24.5 ± 4.6	22.3 ± 5.5	0.45 (overall)
HbA1c (31–44 mmol/mol)	34 (32–36)	44 (36–45)	36 (32–42)	45 (44–55)	**<0.001*** (overall)
Pancreatic magnetic resonance imaging					
Gland volume (ml)	63.2 ± 17.0	63.2 ± 15.6	-	-	0.99
	-	-	67.2 ± 13.6	59.5 ± 17.0	0.53 (overall)
T1 relaxation time (ms)	823 (785–852)	868 (782–909)	-	-	0.07
	-	-	782 (769–867)	909 (868–1085)	**<0.001* (overall)**
MRE-derived stiffness (kPa)	1.23 (1.12–1.28)	1.31 (1.23–1.36)	-	-	**0.01***
	-	-	1.30 (1.22–1.33)	1.32 (1.24–1.43)	**0.03*** (overall)
P roton density fat fraction (%)	3.0 (1.9–4.8)	3.5 (2.2–6.9)	-	-	0.26
	-	-	2.5 (1.9–5.1)	4.5 (2.5–8.1)	0.16 (overall)

Data are expressed as mean±standard deviation or median and (range).
[a]p-value is results from t-test between healthy controls and carriers of m.3243A>G, unless "overall" is stated, which refers to analysis of variance between three groups (healthy controls, non-diabetic and diabetic carriers of m.3243A>G).
HbA1c - hemoglobin A1C; ml - milliliter; ms - milliseconds; MRE - magnetic resonance elastography; kPA - kilopascal.

Conclusion: While there was no pancreatic atrophy or increased fat content in m.3243A>G carriers on MRI, T1 relaxation time and MRE-derived stiffness were slightly higher in diabetic carriers. This may indicate mild fibrotic pancreatic changes secondary to diabetes, comparable to MRI findings in type 1 and type 2 diabetes (3).

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P08.040.C Late-onset epi-CblC: Correlation of *MMACHC* promoter methylation and *PRDX1*expression in patient blood and fibroblasts

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Background: Mutations in the *MMACHC* gene leading to AR combined methylmalonic aciduria and homocystinuria (cblC). Additionally, specific mutations in the downstream inversely oriented *PRDX1* gene resulting in aberrant *PRDX1* transcripts overlapping *MMACHC* promoter lead to its methylation and expression downregulation (epi-CblC). We identified a patient with unusually late-onset epi-CblC type having compound heterozygous pathogenic *MMACHC*:c.271dup and novel *PRDX1*:c.599G>A variant causing aberrant splicing. We detected increased methylation of the *MMACHC* promoter in the patient blood compared to fibroblasts. We aimed to determine whether the observed difference in promoter methylation between the tissues is related to expression of the PRDX1 gene, which could contribute to explain the late-onset in the patient.

Material and Methods: Pyrosequencing and methyl-specific qPCR of bisulfite-treated DNA from blood and fibroblasts of the patient and two healthy controls were performed to evaluate the methylation level of the *MMACHC* promoter. The gene expression of *MMACHC* and *PRDX1* was assessed by qPCR.

Results: We confirmed that *MMACHC* promoter methylation was increased in the patient blood (35%) compared to fibroblasts (5%) by pyrosequencing and qPCR. The *MMACHC* expression was significantly decreased in the patient blood compared to blood of healthy controls, while the expression in fibroblasts was compar-

able with controls. The *PRDX1* gene expression was higher in the patient blood when compared to healthy controls, but was at the same level as expression in the patient fibroblasts.

Conclusion: We did not confirm the association between *PRDX1* expression and level of the *MMACHC* promoter methylation in the patient blood and fibroblasts.

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P08.041.D *EARS2*-related mitochondrial encephalopathy: insight into the disease mechanism

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Background: *EARS2* is a nuclear gene encoding for the mitochondrial glutamyl-tRNA synthetase, a mtDNA translation enzyme responsible for the esterification reaction of glutamate with its cognate tRNA. Autosomal recessive *EARS2* variants lead to mitochondrial encephalomyopathy. Our project aims to understand the disease mechanism of *EARS2* disease to explain the clinical variability of patients and identify targets for therapy.

Material and Methods: a family with one proband and one unaffected sibling, both carrying recessive pathogenetic variants in the *EARS2* gene (c.484C>T; c.600C>A), was identified by whole exome sequencing. Muscle biopsy and fibroblast cell lines of both siblings were analysed with molecular genetics, biochemical, and electron microscopy studies.

Results: mitochondrial respiratory chain activities in muscle homogenate revealed an abnormal increase of citrate synthase in the unaffected sibling demonstrating a compensatory mechanism preventing the manifestation of the disease, whereas a multiple OXPHOS deficiency was identified in the symptomatic patient. Complex IV and V subunit protein levels were decreased in patients' fibroblast cell lines compared to controls. In vitro S^{35} L-methionine labeling assay demonstrated a severe reduction of the de novo mitochondrial protein synthesis in both patients. Oxidative stress and reduction of the mitochondrial mass were also present as well as impairment of the mitochondrial bioenergetics, network, and dynamics. All the abnormalities were more pronounced in the symptomatic sibling. Preliminary data from RNA-bulk sequencing revealed deregulation of mitophagy and OXPHOS metabolism pathways.

Conclusion: our results suggest an interplay between mitochondrial dynamics, translation, and oxidative stress in the disease mechanism of EARS2 defect.

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P08.042.A NGS detection of mtDNA multiple deletions uncovers gene-specific patterns

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Background: Mitochondrial DNA (mtDNA) is maintained, transcribed, and replicated by enzymes encoded by nuclear DNA. Pathogenic variants in genes forming the mtDNA replisome are linked to conditions ranging from ptosis and isolated myopathies to severe encephalomyopathies. These disorders are often linked to deleted mtDNA molecules accumulation in post-mitotic tissues (eg skeletal muscle) or, more rarely, to mtDNA copy number depletion.

Material and Methods: We examined the abundance and characteristics of multiple mtDNA deletions in skeletal muscle biopsies from patients with a myopathy/encephalomyopathy phenotype, carrying pathogenic variants in genes involved in mtDNA maintenance. Whole mtDNA was sequenced via next-generation sequencing (NGS) after target enrichment from total genomic DNA. Deletions and duplications were mapped by identifying reads with breakpoints using the MitoSAlt tool.

Results: The abundance of mtDNA deletions varied significantly across patients, with the highest levels in *POLG* cases and the lowest in *OPA1* cases. Most deletions were mapped to the major arc, but a significant number were identified in the minor arc, particularly in *POLG* cases. Although mtDNA duplications are less reported, significant levels were found in a subset of patients, including those with *DGUOK*, *MGME1*, *RNASEH1*, *TOP3A*, and some *POLG* and *TWNK* cases. These findings suggest novel mechanisms in mtDNA deletion formation.

Conclusion: This study offers the first NGS-based characterization of multiple mtDNA deletions in skeletal muscle from patients with mitochondrial replisome defects. Specific deletion patterns linked to some genetic defects were identified, highlighting the potential of deletion mapping for molecular diagnostics and for understanding replisome proteins' function.

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P08.043.B CLINICAL AND BIOCHEMICAL ANALYSIS OF INFANTS WITH ABNORMAL NEWBORN SCREENING FOR KRABBE DISEASE WITH FOUR *GALC* VARIANTS

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Background: A challenge in using molecular genetic testing as part of newborn screening (NBS) for Krabbe disease (KD) is predicting disease risk given *GALC* variants of uncertain significance or conflicting reports about pathogenicity.

Material and Methods: We analyzed biochemical genetic results and clinical outcomes for infants identified by the New

York NBS Laboratory or Mayo Clinic's Biochemical Genetics Laboratory and found to have at least one of the following *GALC* variants: c.334A>G (p.T112A), c.956A>G (p.Y319C), c.1901T>C (p.L634S), or c.349A>G (p.M117V).

Results: All children homozygous for p.T112A or compound heterozygous for p.T112A and a pathogenic *GALC* variant were clinically asymptomatic (n = 73). Five children with the p.Y319C variant in the compound heterozygous or homozygous state developed late infantile KD and received a bone marrow transplant. 35 other children with the p.Y319C variant in the compound heterozygous or homozygous state were asymptomatic. One of 26 children who are compound heterozygous for p.L634S developed infantile KD. Of 12 children with a *GALC* genotype including p.M117V, two compound heterozygous patients developed infantile KD.

Conclusion: Evaluating the long-term clinical outcomes and biochemical findings of children with commonly identified *GALC* variants and an abnormal NBS for KD is useful to understand genotypes of uncertain significance. Here we provide an update on our understanding of the genotype/phenotype correlation of KD with the goal of reducing unnecessary anxiety and improving long-term follow up of children at risk to develop KD.

C.O.I. Disclosure:

D. Matern: None. **A.L. White:** None. **J.J. Orsini:** None. **D.S. Rajan:** None. **C.A. Saavedra:** None.

P08.044.C Altered mitochondrial transcripts in Primary Mitochondrial Myopathies characterized by mtDNA deletions: an RNA-seq approach

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Background: Primary mitochondrial myopathies (PMM) are a group of heterogeneous disorders characterized by skeletal muscle involvement due to genetic defects in mitochondrial DNA (mtDNA) or nuclear DNA. Single large or multiple mtDNA deletions are common findings in PMM, however, the effects at transcriptional level of this type of alterations are not fully elucidated. This study aims to investigate gene expression alterations and gene fusion events in patients affected by single large-scale or multiple mtDNA deletions.

Material and Methods: we studied total RNA samples isolated from muscle biopsies of PMM patients affected by single (N = 30) and multiple (N = 15) mtDNA deletions, and of 10 controls. RNA sequencing (RNA-seq) was performed on Novaseq platform (Illumina), differential gene expression analysis and identification of fusion events were conducted on mt-mRNA transcripts.

Results: bulk RNA-seq revealed differentially expressed mtDNA-genes in patients with single or multiple mtDNA deletions compared to controls. In single deletions, expression levels of genes within the deletion decreased proportionally to deletion heteroplasmy. In multiple deletions, reduced gene expression showed a weaker association with cumulative heteroplasmy. Gene fusion events caused by deletions were also analyzed, identifying primarily involved genes.

Conclusion: this study provides novel insights into the mitochondrial transcriptome alterations associated with mtDNA deletions in PMM patients. Bulk RNA-seq allowed for the identification of mtDNA DEGs and gene fusion events in muscle tissue. These results may contribute to a better understanding of the molecular mechanisms underlying PMM and of their clinical heterogeneity.

C.O.I. Disclosure:

A. Legati: None. **R. Izzo:** None. **M. Barresi:** None. **E. Moresco:** None. **A. Nasca:** None. **E. Lamantea:** None. **C. Lamperti:** None. **D. Ghezzi:** None.

P08.045.D Biallelic or X-linked variants in nuclear genes coding for mitochondrial Complex I subunits are a frequent cause of isolated and syndromic optic atrophy

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Background: The genetic landscape of inherited optic neuropathies (ON) is continuously expanding to include several genes encoded in both nuclear and mitochondrial DNA, therefore encompassing a wide range of inheritance patterns. Occasionally, a few pathogenic variants in mitochondrial respiratory Complex I (CI) genes have been identified in optic neuropathy cases, besides the more typical severe presentation of CI deficiencies.

Material and Methods: We investigated CI genes through NGS screening to identify possibly causative variants in patients with optic atrophy. Those who were found to carry pathogenic possibly causative variants underwent additional clinical evaluations, including MRI and neurological examinations.

Results: We identified pathogenic or likely pathogenic variants in 31 patients from 23 families, with biallelic or hemizygous variants in 11 different CI-related genes. These variants span all major types of peptides involved in the structure of CI, including 3 core subunits (*NDUFS7*, *NDUFV1*, *NDUFV2*), 4 accessory subunits (*NDUFA1*, *NDUFA10*, *NDUFA12*, *NDUFB11*), and 4 assembly factors (*NDUFAF2*, *NDUFAF3*, *NDUFAF4*, *NDUFAF8*). Notably, defects in core CI subunits lead to isolated optic atrophy, while defects in accessory CI subunits and assembly factors lead to a spectrum ranging from isolated to syndromic optic atrophy. In some cases, the disease presentation with acute onset of vision loss allowed us to link novel genes to the recessive Leber hereditary optic neuropathy (LHONAR) phenotype.

Conclusion: Our case series broadens the genetic landscape of ON and LHONAR phenotypes, highlighting the pivotal role of

nuclear CI genes in ON pathogenesis and their so far underestimated prevalence in ON molecular diagnosis.

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P08.046.A Transaldolase deficiency (Eyaid syndrome): Natural history of hepatic involvement

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Background: Transaldolase deficiency is an autosomal recessive disorder that impacts the pentose phosphate pathway, a crucial pathway causing oxidative stress and multisystem failure, primarily affecting the liver, which is responsible for the mortality associated with this disorder. This study aims to clarify the clinical variability and hepatic manifestations of this uncommon condition through a comprehensive case series.

Material and Methods: We analyzed 21 patients diagnosed with transaldolase deficiency, focusing on their clinical presentations, familial patterns, and outcomes post-intervention. Data were collected via medical records, genetic testing, imaging, and liver biopsies. Patients were categorized based on familial occurrence and severity of liver disease.

Results: The study revealed a high incidence of familial clustering, with consanguinity noted in several families, highlighting the genetic predisposition. Hepatosplenomegaly was universal, with varying degrees of liver fibrosis and cirrhosis. Two patients underwent liver transplantation with successful outcomes, indicating its potential as a therapeutic option. Other manifestations included congenital heart defects, metabolic disorders, and in some cases, early mortality due to severe liver failure.

Conclusion: This case series underscores the phenotypic heterogeneity of transaldolase deficiency, emphasizing the need for early diagnosis, genetic counseling, and considering liver transplantation in severe cases. The findings advocate for a multidisciplinary approach in managing this condition, with a focus on long-term prognosis and post-transplant stability.

C.O.I. Disclosure:

N.S. Almutairi: None. **A. Al Shamsi:** None. **W. Eyaid:** None.

P08.047.B A case of neonatal lactic acidosis: investigating *SUCLG1*-related mitochondrial DNA depletion syndrome

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Background: Variants in *SUCLG1* disrupt succinyl-CoA ligase enzyme function, impairing mitochondrial DNA recycling and energy generation, resulting in mitochondrial DNA depletion. Symptoms range from severe hypotonia and developmental delay to fatal infantile lactic acidosis, depending on enzyme activity loss.

Material and Methods: Whole-exome-sequencing, tandem-mass-spectrometry and gas-chromatography-mass-spectrometry were used for diagnostic work-up.

Results: A neonate born to consanguineous parents underwent prenatal trio-exome diagnostics due to an SGA fetus with short femurs, suspected Fallot's tetralogy, and vermis hypoplasia. Initial analysis did not identify (likely) pathogenic variants. After birth, the infant developed severe lactic acidosis (30 mmol/L), metabolic decompensation, and liver failure, necessitating intensive care. Metabolic profiling revealed elevated propionylcarnitine levels and related ratios in acylcarnitine analysis, alongside markedly increased urinary concentrations of lactate, 3-hydroxypropionic acid, methylcitrate, and methylmalonic acid. The presence of methylglutaconic acid and 3-hydroxy acids suggested mitochondrial dysfunction or mitochondrial DNA depletion syndrome, particularly involving *SUCLG1* or *SUCLA2*. Reanalysis of the trio-exome identified a homozygous variant of uncertain significance (c.719T>G, p.(Leu240Arg), VUS, class 3) in *SUCLG1*, correlating with the clinical phenotype. Classification using ACMG criteria (PP4-moderate, PM2-supporting, PP3-moderate) yielded a VUS score of 5, indicating a "hot" VUS requiring additional evidence for definitive interpretation.

Conclusion: This case highlights the importance of interdisciplinary diagnostics and integrated omics in identifying disease mechanisms in critically ill patients. Planned steps include genetic testing of an affected aunt with neonatal losses and functional studies to validate the variant's pathogenicity. As the variant remains a VUS, further evidence is required to confirm its disease association.

C.O.I. Disclosure:

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P08.048.C Severe recurrent rhabdomyolysis with alpha-methylacyl-CoA racemase (AMACR) deficiency

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Background: AMACR deficiency is a rare autosomal recessive peroxisomal disorder, resulting in impaired conversion of branch-chain fatty acid pristanic acid and C_{27}-bile acid intermediates, dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA), to their corresponding (S)-isomers. Clinical presentation can vary with age. Adult onset presentation is characterized by sensorimotor neuropathy, pigmentary retinopathy, seizures, ataxia, and cognitive decline. Children can present with transaminitis, and neonatal cholestasis. Rhabdomyolysis at any age is a rare presentation.

Case Report: We report a 46 year old male with severe recurrent episodes of rhabdomyolysis, beginning in adolescence. Rhabdomyolysis has become more frequent with age, and requires less exertion to precipitate an episode, with hospitalization roughly every 2–3 months over the past several years. Between episodes, he experiences chronic myalgia and muscle cramps. A novel homozygous variant in the AMACR gene, was predicted to be damaging by in silico analysis. Biochemical analysis identified markedly elevated pristanic acid, THCA and DHCA, consistent with a diagnosis of AMACR deficiency. Whole exome sequencing did not identify an alternate explanation for his episodes of rhabdomyolysis.

Conclusion: Rhabdomyolysis has only been reported in three previous published cases of AMACR deficiency. Two of the patients were on antipsychotic medications, which is not the case for our patient. Our patient follows a low phytanic acid/ pristanic acid diet, however, this has not altered his clinical course. Our case provides further evidence that rhabdomyolysis is a clinical presentation associated with AMACR deficiency, in the absence of the more well described neurologic features of this condition.

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S. Goobie: None. **A. Vandersteeen:** A. Employment (full or part-time); Significant; Creyon Bio.

P08.049.D Novel FH gene variant with intrafamilial variability: expanding the phenotype spectrum of a rare disease

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Background: Fmarase Hydratase (FH) is a critical enzyme in the Krebs cycle, playing a pivotal role in cellular energy production. Biallelic pathogenic variants in the FH gene lead to fumarase deficiency (FMRD), also known as fumaric aciduria, a rare autosomal recessive metabolic disorder primarily affecting the nervous system. This study presents a consanguineous family with five affected individuals harboring a novel FH variant of uncertain significance (VUS), demonstrating biochemical confirmation and significant intrafamilial phenotypic variability.

Material and Methods: A retrospective analysis was conducted on 13 patients who underwent genetic testing and urine organic acid analysis. Clinical data were reviewed and compared with existing literature.

Results: Whole exome sequencing (WES) identified a novel homozygous FH variant (Chr1:241504102, NM_000143.4, c.1048C>G, p.(Arg350Gly)), initially classified as a VUS. Segregation analysis confirmed its presence in affected family members. Urine organic acid analysis via mass spectrometry revealed elevated fumaric acid levels in homozygous patients, corroborating the biochemical diagnosis of FMRD. Notably, affected individuals exhibited a delayed-onset phenotype, with symptoms such as psychomotor retardation, hypotonia, and seizures manifesting during puberty, contrasting with the typically severe early-onset presentation and high mortality associated with FMRD.

Conclusion: This report underscores the expanding phenotypic spectrum of FMRD, emphasizing intrafamilial variability and a delayed-onset clinical course. The findings highlight the necessity of integrating biochemical assays with genetic testing to establish variant pathogenicity in rare metabolic disorders. This case contributes to the growing understanding of FMRD and reinforces the importance of comprehensive diagnostic approaches in managing rare genetic diseases.

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P08.051.B A novel*NADK2*frameshift variant in a patient with mitochondrial NAD kinase deficiency: Expanding the genotypic spectrum

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Background: Mitochondrial the nicotinamide adenine dinucleotide (NAD) kinase deficiency is a rare autosomal recessive disorder of the nicotinamide adenine dinucleotide phosphate (NADPH) biosynthesis that presents with variable severity and can cause dienoyl-CoA reductase deficiency (DECRD, OMIM #616034). This disorder is caused by variants in NAD kinase 2, mitochondrial (*NADK2*, OMIM *615787), located on chromosome 5p13.2. In this study, we present a currently 12-year-old patient who is under follow-up for growth retardation, global developmental delay, microcephaly, and thin corpus callosum. The patient's laboratory work-up including metabolic tests have been normal.

Material and Methods: The whole exome sequencing (WES) was performed.

Results: WES analysis identified a homozygous c.1124dup variant in the NADK2 gene. The detected variant was classified as "likely pathogenic" according to American College of Medical Genetics (ACMG) guidelines and was not reported in healthy population databases.

Conclusion: This case highlights the importance of WES analysis in elucidating the genetic etiology of patients with neurometabolic disorders. Our findings unveil that the molecular pathogenesis in this patient may be driven by NADPH-dependent processes rather than hyperlysinemia and DECRD.

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P08.052.C Investigation of Fatty Acid β-Oxidation Defects in Rio Grande do Sul, a Southern State of Brazil

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Background: Fatty acid β-oxidation defects (FAODs) are a group of disorders affecting the fatty acid transport chain and mitochondrial β-oxidation. These conditions impact energy homeostasis, primarily in the liver, heart, and skeletal muscles, and often present with multi-system involvement and potentially life-threatening manifestations. FAODs are inherited as autosomal recessive disorders with variable presentation, manifesting either in the neonatal period or later.

Material and Methods: This study aimed to clinically characterize FAOD patients in Rio Grande do Sul, Brazil, focusing on clinical, biochemical, genetic, and therapeutic aspects. A retrospective study was conducted using data from medical records submitted by physicians through an electronic form. Ethical approval was obtained.

Results: Sixteen patients were included, 62.5% female, with a mean age of 19.2 years. The majority (75%) were born in Rio Grande do Sul. Common signs and symptoms included hypoketotic hypoglycemia (81.3%), liver dysfunction (68.8%), and both seizures and vomiting (56.3%). The most frequent diagnoses were Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD, 31.3%) and Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD, 18.8%). Average time to diagnosis was 5.14 years, predominantly made during a metabolic crisis (56.3%), with confirmation through genetic analysis (87.5%) and acylcarnitine profile (81.3%). Nutritional therapy was provided to 93.8% of patients, including carnitine supplementation (50%), triheptanoin (43.8%), and medium-chain triglycerides (25%). There were two recorded deaths (12.5%).

Conclusion: FAODs are multi-systemic and heterogeneous, with a prolonged diagnostic odyssey. Clinical management highlights the importance of nutritional and multidisciplinary therapy. Further research, professional training, and public policies for early diagnosis, including neonatal screening, are essential.

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P08.053.D French guidelines for the diagnosis, follow-up and management of individuals with Smith-Lemli-Opitz syndrome and establishment of a French natural history cohort

Paul Rollier[1,2], *Sylvie Odent*[1,2], *Thierry Levade*[3], *Cécile Pagan*[4], *Antonin Lamaziere*[5], *Pascale Benlian*[6], *Charlotte Dubucs*[7], *François Labarthe*[8], *François Maillot*[9], *Muriel Girard*[10], *Stéphane Decramer*[11], *Dries Dobbelaere*[12,13], *Fabien Labombarda*[14], *Annick Toutain*[15], *Chloé Quélin*[1,16], *Ophelie Boutfol*[1,17]

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Background: Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive polymalformative syndrome caused by 7-dehydrocholesterol reductase (*DHCR7*) deficiency. Its natural history remains poorly understood, with no international consensus on treatment or management. Alongside the creation of a French SLOS cohort in parallel to an European cohort, a national diagnosis and care protocol (PNDS) has been established to standardize disease management.

Material and Methods: A group of expert physicians (from national rare disease networks) was formed to review the literature on SLOS. The cohort, based on the French rare diseases database BaMaRa, was set up in collaboration with ERN MetabERN (UIMD) to monitor retrospectively and prospectively SLOS individuals across French centers.

Results: Collegial discussions led to consensus on key clinical signs of SLOS, aiding non-specialists in recognizing the syndrome, and establishes monitoring guidelines. Cholesterol supplementation has shown no adverse effects in normalizing blood cholesterol levels, despite the lack of clear evidence for survival improvement. In contrast, statins have an unfavorable benefit-risk ratio, offering no benefit and potential adverse effects. Beyond genetic counseling, extended family screening and the identification of *DHCR7* heterozygotes are important. Mouse studies suggest that some psychotropic drugs (aripiprazole, trazodone) which inhibit DHCR7 can cross the placental barrier and may induce pseudo-SLOS. The use of these drugs should be avoided a few weeks before and during pregnancy if either parent is a SLOS carrier. Ongoing cohort data collection will refine these recommendations.

Conclusion: The first recommendations for surveillance and management of SLOS have been developed and validated by French experts.

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P08.054.A Association between variants of the LEP, LEPR, and FTO genes with obesity in adults from the Colombian Caribbean (Cartagena de Indias, Colombia)

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Background: Obesity is a public health issue influenced by genetic and environmental factors. Genes such as LEP, LEPR, and FTO have been associated with an increased risk of obesity in various populations. Cartagena de Indias, with its mixed

population and unique ethnic characteristics, represents a relevant context for study.

Material and Methods: A case-control study was conducted with adults from the Colombian Caribbean who are part of the Cartagena Cohort Study (CaReS) in Cartagena de Indias, Colombia. Risk variables were analyzed. Polymorphic variants (Single Nucleotide Polymorphisms, SNPs) in the genes LEP (rs7799039), LEPR (rs1137101), and FTO (rs9939609) were genotyped. Allelic and genotypic frequencies were calculated by direct counting, and Hardy-Weinberg equilibrium was assessed. To compare case and control groups, null hypothesis tests were applied.

Results: A total of 150 cases and 150 controls (with and without obesity, respectively) were included. Variants in the FTO, LEP, and LEPR genes were found to be associated with the risk of developing obesity. The absence of the AA genotype in FTO, AA in LEP, and AG in LEPR increased the risk, whereas the presence of AA (LEP) was associated with a lower risk of developing obesity. Significant differences were found in the genotypic distribution of the SNP rs9939609 (FTO) between case and control groups ($p < 0.05$).

Conclusion: The polymorphic variants of the FTO (rs9939609), LEP (rs7799039), and LEPR (rs1137101) genes are suggested to be associated with the risk of developing obesity in this population. Further analyses are needed to confirm these findings.

C.O.I. Disclosure:

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P08.056.C Whole exome sequencing has identified genetic risk factors contributing to non-syndromic early-onset childhood obesity in consanguineous families

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Background: Obesity is defined as abnormal fat accumulation that leads to comorbidities presenting life-threatening risks. Childhood obesity has been acknowledged to elevate mortality risk due to type-2-diabetes mellitus (T2DM), cardiovascular diseases, and cancer. We aimed to identify candidate genes for obesity utilizing independent consanguineous families from Turkiye.

Materials and Methods: Three families having at least one obese child were recruited with body mass indexes (BMI) calculated in available members. Peripheral DNA obtained from all recruits was subjected to whole exome sequencing (WES), followed by bioinformatic analysis on the Genomize-SEQ Platform. Variant filtering for minor allele frequency (MAF)<0.01 in all normal populations was applied, and candidates were confirmed by Sanger sequencing of family members.

Results: A total of 16 individuals were recruited. WES identified a shared variant in the *ATXN3* gene across all affected family members. Among additional genetic variants detected in different families, two were in *SH2B1* and *ADIPOQ* genes; previously associated with obesity. Furthermore, five novel heterozygous variants in *ANKK1*, *NEGR1*, *OGDH, ABCB1*, and *GSK3B* genes were discovered and predicted to lead to excessive fat accumulation and dysregulation of energy metabolism in individuals.

Conclusion: Our results suggest the cumulative impact of variants in multiple genes leading to the early-onset obesity observed in individuals from disparate families. The shared variant in the *ATXN3* gene is a strong candidate for obesity, which will be screened in a sporadic cohort prior to functional analyses. Regular follow-up was recommended to the families, along with embracing a more active lifestyle and healthier eating habits.

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H.B. Olgun Celebioglu: None. **A.P. Ozturk:** None. **S. Poyrazoglu:** None. **F.N. Tuncer:** None.

P08.057.D Unusual case of MPS I diagnosed at the age of 28 years old

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Background: We present a case of a 28-year-old male, who was referred for genetic consultation by the cardiologist, due to aortic regurgitation III^0, bicuspid aortic valve, and mitral regurgitation II^0 with the suspicion of Loeys-Dietz's syndrome. Physical examination revealed a narrow forehead, synophrys, big nose, mouth, and ears, blurry vision at night, short neck, contracture and deformity[1] of upper and lower limbs, other bone deformities, and umbilical and inguinoscrotal hernias. The 32-year-old brother of the patient who was also present at the genetic consultation, has similar symptoms (similar facies, upper and lower limb contracture and deformity, umbilical hernia, and cardiac abnormalities) indicating a possibility of a familial case of MPS I[2].

Methods: The type of genetic testing was the "connective tissue" disease panel. Analysis was performed using Next-Generation Sequencing (NGS)[3].

Results: NGS identified two heterozygous mutations in the IDUA gene (chr4:987085A>C, chr4:987915C>T), consistent with MPS I (AR), and an additional heterozygous variant of uncertain significance (VUS) in the COL9A3 gene.

Conclusion: This case underscores the familial nature of Mucopolysaccharidosis (MPS) I, as evidenced by the similar clinical features observed in both the 28-year-old patient and his 32-year-old brother. The patient's heterozygous mutations in the IDUA gene support the MPS I diagnosis and emphasize the need for additional genetic testing on the brother to determine his risk of the condition. Such patients mainly die at an early age, especially in the absence of appropriate treatment. This case is unusual as both the phenotype and genetic findings are atypical for MPS I.

C.O.I. Disclosure:

L. Barseghyan: None. **S. Muradyan:** None. **T. Sarkisian:** None. **A. Gasparyan:** None.

P08.058.A Metabolic syndrome and effect of gene polymorphisms*ADIPOQ* rs266729, rs17300539, and rs2241766, *AGT* rs699 and rs4762, *AGTR1* rs5186, *AGTR2* rs11091046, *ApoC-III* rs2854116 and rs2854117, *NR3C1* rs10052957 and rs41423247, and *GNB3* rs5443 gene polymorphisms

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Turkey, [4]Department of Hematology, Istanbul Basaksehir Cam and Sakura City Hospital, Istanbul, Turkey.

Background: Metabolic syndrome (MetS) is defined as a metabolic dysfunction characterized by the co-occurrence of multiple risk factors. The aim of our study was to elucidate the effects of ADIPOQ rs266729, rs17300539, and rs2241766, AGT rs699 and rs4762, AGTR1 rs5186, AGTR2 rs11091046, ApoC-III rs2854116 and rs2854117, NR3C1 rs10052957 and rs41423247, and GNB3 rs5443 gene polymorphisms on MetS.

Material and Methods: The study included 200 patients who met the diagnostic criteria for MetS and 100 healthy controls. Variant analyses were performed using PCR-RFLP from genomic DNA samples.

Results: ADIPOQ rs17300539 GA, AGT rs699 TT, AGTR1 rs5186 AC, and AGTR2 rs11091046 CC genotypes were more frequently observed in patients diagnosed with MetS compared to healthy controls ($p < 0.05$, for all). MetS patients with AGT rs699 TT genotype had a higher incidence of hypertension compared to individuals with CC/CT genotype of the same polymorphism ($p = 0.047$). Individuals with ADIPOQ rs17300539 AA/AG and NR3C1 rs41423247 CC genotypes were found to have higher mean waist circumferences compared to others ($p < 0.05$, for all). Individuals with ADIPOQ rs17300539 AA/AG, NR3C1 rs10052957 GG, and AGT rs4762 GA/AA genotypes exhibited significantly higher incidence of nephropathy. Individuals with ADIPOQ rs17300539 AA/AG and NR3C1 rs10052957 GG genotypes had significantly higher incidence of retinopathy compared to others.

Conclusion: Our study, which has yielded numerous novel findings regarding MetS and gene polymorphisms, will illuminate future research.

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P08.059.B Newborn novel monoallelic variants in *ACADVL* gene correspond to the enzymatic activity reduction: implication for pathogenetic definition

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Background: Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) is a rare autosomal recessive inborn error detected by newborn screening (NBS), which is caused by defects in *ACADVL* gene. This leads to the abnormal accumulation of long chain acylcarnitines and consequently to cardiac, muscle and liver disfunctions. Our aim was to investigate the correspondence between residual enzymatic activity (REA) and 3 novel missense variants in VLCADD heterozygous newborns.

Material and Methods: Dried blood spots collected from newborns were analyzed at the NBS Laboratory, Buzzi Children's Hospital, Milan, Italy. Three newborns with very slight increase in C14, C14:1, C14:2 acylcarnitines were referred for clinical evaluation and follow-up. Whole exome sequencing was performed on DNA from DBS. REA was detected in lymphocytes.

Results: One subject with a REA = 29% showed the c.1048G>A (p.Gly350Ser) classified as VUS by ClinVar and AlphaMissense and likely pathogenic by ACMG. The second neonate had the c.1291G>C (p.Asp431His) variant classified as VUS by ClinVar and ACMG and pathogenic by AlphaMissense and a REA = 25%. The third one had the c.757G>A (p.Gly253Arg) classified by ClinVar as VUS and likely pathogenic by ACMG and AlphaMissense with a REA = 28%. All variants were within the protein catalytic domain.

Conclusion: We report three novel heterozygous missense variants in the *ACADVL* gene corresponding to REA levels lower than 30%. This may be due to the variants that modify the aminoacids within the protein catalytic domain. It might be important to monitor these subjects since some heterozygous with severe reduced REA could develop disease-related symptoms overtime.

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P08.060.C Unique Genetic Variants and Population Dynamics of Phenylketonuria and Wilson's Disease in Ecuador

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Background: Phenylketonuria (PKU) and Wilson's disease are rare metabolic disorders with profound public health implications in Ecuador. PKU results from pathogenic PAH variants causing phenylalanine accumulation, while Wilson's disease arises from ATP7B mutations affecting copper metabolism. This study explores the genetic landscape of these disorders, focusing on unique variants and clinical challenges in Ecuador's diverse population.

Methods: Next-generation sequencing identified PAH and ATP7B variants in Ecuadorian patients. Population dynamics, including founder effects and geographic clustering, were analyzed. Barriers to effective management were assessed through surveys and evaluations of dietary and treatment resources.

Results: Fifteen PAH variants were detected in PKU patients, with c.754C>T (p.Arg252Trp) being the most prevalent. The c.441+5G>T variant was concentrated in Papahurco, an isolated population, reflecting a founder effect. For Wilson's disease, rare variants such as p.M685* and p.M671* were common, while the globally frequent p.H1069Q was absent. Limited access to low-phenylalanine foods, supplements, and treatments, particularly in rural areas, highlights systemic disparities.

Conclusions: Ecuador's unique genetic variants necessitate population-specific diagnostic and treatment approaches. Accessible nutritional resources are essential for PKU management, while early genetic testing and improved access to Wilson's disease treatments can mitigate severe outcomes. These findings underscore the need to integrate genetic diversity into global frameworks for rare disease management.

C.O.I. Disclosure:

V.I. Romero Aguilar: None.

P08.061.D Double the trouble: dual diagnosis in Fabry disease may contribute to atypical phenotypes

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Background: Dual (or multiple) molecular diagnoses involve more than one clinical diagnosis and more than one genetic locus, each segregating independently. The obvious significance of Dual Genetic Disorders (DGD) has direct practical implications. From a scientific point of view, case reports that claim "broadening" or "extension" of a particular phenotype for a single-gene disorder should be viewed with caution. In an extreme scenario, the clinical recognition of a "novel syndrome" may turn out to represent DGD for two previous known disorders Material and Methods: clinical and molecular features of a cohort of Brazilian patients with Fabry disease harboring a second pathogenic mutation for another disease.

Results: Among the 340 sequential DNA samples submitted to investigation of hypertrophic cardiomyopathy, small fiber pain with recurrent abdominal pain and progressive nephropathy of unknown cause, a molecular diagnosis involving a mendelian disease gene related to the clinical phenotype at the time of referral was reported for 60% of patients. Two or more molecular diagnoses were reported for 68 patients (20%), including 20 Fabry patients. Some of the atypical features in the Fabry cohort patients were prolonged attacks of abdominal pain even after enzyme replacement therapy (two Fabry patients with acute hepatic porphyrias), recurrent strokes in a patient with Fabry disease and COL4A1 mutation, complex cardiomyopathy in two patients with Fabry disease (one with PTPN11 mutation and the other with TTR-amyloidosis), among others.

Conclusion: Diagnostic next generation sequencing for multiple genes (massive parallel sequencing) affords opportunities for providing insights into relationships between multilocus genomic variation and disease.

C.O.I. Disclosure:

C. Lourenco: None. **A. Queiroz:** None. **J.M. Fonseca:** None.

P08.062.A Associations and interactions of clinical and genetic variables for Type 2 Diabetes Mellitus in the Mexican population

O. M. Ramirez[1], *C. Martínez-Lara*[1], *L. Casas-Avila*[2], *M. Ramos-García*[1], *A. D. Salazar-Piña*[1], *A. Hidalgo-Bravo*[3], *C. Castro-Hernández*[4], *I. Barrera-Molina*[1];

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Background: Eating disorders are complex mental health conditions characterized by abnormal eating habits that negatively impact a person's physical and mental health. Risky eating behaviors are closely related to risk factors such as BMI, sex, depression, and anxiety.

Aim: The objective of this study is to determine the association of gene methylation status RLEP, LEP, FTO, BDNF, and COMT with the risk factors for eating disorders in Mexican university students.

Material and Methods: An analytical cross-sectional study was carried out. Data on risky eating behaviors, anxiety, and depression were collected using self-applicable questionnaires EAT, BAI, and BECK, respectively. The methylation of the genes was determined by MSP-PCR.

Results: We included 83 individuals, 61.4% women, and 38.6% men. Interestingly, our results showed an association between the risk of eating disorder with anxiety and/or depression. When we determine the interaction between genes and risk factors, we find that the greater the number of risk variables, the greater the association of presenting risky eating behaviors in Mexican university students.

Conclusion: We found an association between risk factors, especially depression and anxiety, with risky eating behaviors in Mexican university students.

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P08.063.B Case Report - Leber's Hereditary Optic Neuropathy

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Background: This report presents the case of a boy with early-onset optic atrophy and confirmed diagnosis of Leber's Hereditary Optic Neuropathy (LHON) at age eight through Whole Exome Sequencing (WES). The patient presented with visual impairment since age one, motor difficulties and mild cognitive development delay. Cranial MRI showed symmetrical supratentorial white matter changes. LHON is a rare mitochondrial disorder characterized by optic atrophy and progressive vision loss, typically manifesting in adulthood.

Material and Methods: Genetic investigations included an NGS leukodystrophy panel, followed by single WES and segregation analysis of candidate variants, ultimately followed by trio WES.

Results: After an initial NGS panel for leukodystrophy did not explain a clear cause of the patient's symptoms, single WES identified the mitochondrial homoplasmic variant m.11778G>A in the *MT-ND4* gene, confirmed as maternally inherited by segregation analysis. This variant accounts for over 50% of LHON cases worldwide and is a well-studied pathogenic variant, causing mitochondrial complex I dysfunction. Retinal ganglion cells, with high energy demand, are particularly vulnerable. Clinical findings align with LHON phenotypes. The identified *MT-ND4* variant, however, does not explain the patient's developmental delay. Two years later, trio WES identified a de novo *SPTBN1* variant, associated with developmental delay, intellectual disability, speech disorders and behavioral anomalies. The patient's mother, an asymptomatic carrier of the *MT-ND4* variant, demonstrates incomplete penetrance.

Conclusion: This case highlights the value of mtDNA analysis in pediatric optic neuropathies, where identifying the variant enabled a definitive diagnosis and multidisciplinary care. Emerging therapies, e.g. Idebenone, may improve LHON outcomes.

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P09 Immunology and Hematopoietic System

P09.001.C LINE1 modulate human T cell function by regulating protein synthesis during lifespan

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Ricciardi[1], Sergio Abrignani[1], Carlo Pietrasanta[5], Federica Marasca[1], Beatrice Bodega[1]

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Background: Neonatal T cells form a unique developmental layer, characterized by the absence of memory T lymphocytes and a predominance of naïve T cells. Upon activation, neonatal naïve T cells exhibit a heightened readiness for activation, but the molecular mechanisms behind this rapid T cell mobilization in neonates remain unclear. Our previous study uncovered a novel transcriptional regulatory mechanism in adult naïve CD4+ T cells involving the splicing of LINE1 elements into new transcript variants, which control T cell quiescence and activation state.

Materials and methods: In this study, we isolated naïve CD4+ T cells from enrolled healthy donors across neonatal, adult, and elderly age groups. Using flow cytometry analysis, we evaluate the quiescent and activated states of naïve CD4+ T cells, assessing cell cycle, surface marker expression, and protein synthesis. We also conducted RNAseq to identify gene classes and biological processes affected by the absence of LINE1.

Results: We found that neonatal naïve T cells, upon activation, exhibit faster cell cycle entry, higher energy production, and increased protein synthesis compared to adults. Neonatal naïve T cells show reduced LINE1 levels due to tonic TCR activation by self-antigens, maintaining basal mTORC1 signalling and suppressing LINE1 via PTBP1-mediated splicing suppression. Modulating LINE1 through tonic TCR stimulation affects protein synthesis and cell cycle progression. Additionally, we observed that LINE1 levels increase with age, while protein synthesis rates decline.

Conclusions: Our study identified a novel role for LINE1 in T cell development, highlighting its critical function in human T cell activity during aging.

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P09.002.D Unveiling X-Linked Primary Immunodeficiencies: The Impact of Genetic Testing on Early Diagnosis and Novel Variant Discovery

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Background: Primary immunodeficiencies (PI) are genetic disorders that involve the immune system. The Jeffrey Modell Foundation (JMF) is a global non-profit organization dedicated to improving the diagnosis and treatment of PI. X-linked genetic disorders are particularly significant among PIs, as they primarily affect males, which are often underdiagnosed.

Material and Methods: In this study, supported by JMF, samples from patients suspected of PI were analyzed using next-generation sequencing, targeting 575 immune-related genes.

Results: Among 1,591 individuals tested, 334 cases (21%) were genetically diagnosed. Of these, 65 cases (19.5%) involved X-linked genetic variants. The two most prevalent genes among the X-linked cases were *BTK* and *WAS*, associated with Agammaglobulinemia and Wiskott-Aldrich Syndrome, respectively. Both accounted for 41 genetic diagnoses, representing 12.3% of all positive cases and 63% of the X-linked cohort. Interestingly, among the 41 X-linked diagnostic variants in these genes, 25 had not been previously reported in the clinical literature. Of these, 16 were likely pathogenic truncating variants, while 8 were missense, categorized as strong variants of uncertain significance (SV). Finally, one of the diagnostic variants was a synonymous SV located in the last nucleotide of the exon, predicted to affect splicing.

Conclusion: This study highlights the critical role of early genetic testing in diagnosing PI, while emphasizing the need for greater clinical attention to X-linked PI due to their high prevalence. The significant proportion of novel SV variants highlights the key role of functional studies and segregation analyses to determine their pathogenicity, which would improve and complement diagnostic accuracy.

C.O.I. Disclosure:

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P09.003.A Single cell profiling of peripheral immune cells in Parkinson's disease

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Background: Although Parkinson's disease research has unsurprisingly focused on the neurodegeneration happening in the brain multiple studies are pointing to peripheral immune dysregulation as a key contributor to PD progression. Therefore, we aimed to perform the first comprehensive analysis of the complete peripheral blood mononuclear cells compartment in PD patients utilizing the resolution of single-cell sequencing.

Material and Methods: PBMC were isolated from 14 blood samples from individuals with PD diagnosis, and 10 healthy age-matched control samples. Single-cell libraries were obtained with standard 10X Genomics protocols. Transcriptomic and T cell receptor (TCR) sequencing were run in parallel on the pools to enable the comparison of transcriptomic and immunologic

profiles from single cells. Standard bioinformatic tools were used for processing and annotations.

Results: Broad cluster analysis showed a general increase in activation signaling in PD samples, exhibiting a slight trend of increased monocytes and decreased lymphocytes. Further analysis of myeloid cells revealed an activated $CD14^{+}/CD83^{+}$ monocyte subpopulation, for which differentially expressed genes suggested a heightened inflammatory state in PD. Lymphoid cells analysis also showed increased pro-inflammatory NK56hi cells in PD samples. TCR clonotyping identified PD-specific sequences, with multiple motifs shared across multiple samples, but none were linked to known causative sequences in current annotation databases.

Conclusion: Overall, we present the first comprehensive single-cell atlas of the PBMC compartment, and provide insights into the peripheral immune mechanisms at play in PD pathogenesis. We believe these data could lead to the definition of prognosis gene signatures, and the identification of new therapeutic targets.

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P09.005.C A novel in vitro platform for functional validation of germline variants in clonal haematopoiesis

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Background: Clonal haematopoiesis of indeterminate potential (CHIP) is characterized by somatic mutations in genes associated with haematological malignancies, at >2% allele fraction in asymptomatic individuals. Recent GWAS have identified germline variants (GVs) linked to CHIP risk, but functional validation remains lacking.

Material and Methods: GVs coming from fine mapping of CHIP GWAS were introduced in H1 human embryonic stem cells using CRISPR/Cas9 alone or in combination with CHIP driver mutations. Cells were differentiated in a competitive setting towards haematopoietic lineages and key time points were assayed by 10x Multiome, tracking the edited genotypes by cell barcoding.

Results: Conditionally independent variants with high fine-mapping posterior probabilities were annotated using the Open-Targets V2G score and manually curated. The GV rs62237617 C>T, predicted to be associated with the expression of CHEK2 (a gene regulating the response to DNA damage and associated to CHIP risk), was selected to be tested in our in vitro platform alone or in combination with the common CHIP driver mutation DNMT3A-R882H.

Conclusion: We set up a functional validation platform to explore the possible interplay of GVs and CHIP mutations. This approach can be further expanded to include multiple GVs, enabling a deeper understanding of the role of germline variation in CHIP.

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P09.006.D Defining genetic determinants of T-cell receptor repertoires at single-cell resolution

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Background: Variation in the human leukocyte antigen (HLA) genes encoded by the major histocompatibility complex (MHC) locus is extensive and mediates risk of autoimmune disease and infection response. HLA presents peptides to the T-cell receptor (TCR), which is formed through somatic recombination of Variable (V), Diversity (D) and Joining (J) gene segments. While bulk sequencing has been used to study HLA-TCR associations, single-cell sequencing allows a more granular investigation of these relationships at the individual cell level.

Material and Methods: We analysed 228,588 single T cells from the COVID-19 Multi-omics Blood Atlas (COMBAT) Consortium, integrating TCR repertoire data with HLA genotypes from 100 donors. We used linear regression models to identify associations between genetic variations in the MHC locus and features of the TCR repertoire, including V-gene segment usage, while adjusting for age and sex.

Results: As expected, we observed cell type-specific associations between MHC variants and the TCR repertoire, particularly with TCR alpha chain V- gene segment usage. Notably, amino acid changes in position 13 in HLA-DRB1 are associated with increased usage of TRAV20 in naive CD4+ T cells (beta = 0.738, p = 1.16e-09) compared to central memory CD4+ T cells (beta = 0.268, p = 0.0533).

Conclusion: Our findings demonstrate that HLA genotype influences TCR repertoire in a cell type-specific manner, reinforcing the importance of single-cell resolution analyses to uncover dynamic HLA-TCR associations.

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P09.007.A Early Genetic Diagnosis of ELANE-Related Neutropenia: Insights from a Global Primary Immunodeficiency Screening Program

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Background: Primary Immunodeficiencies (PI) are a group of more than 450 inherited conditions affecting the immune system. Early and accurate diagnosis of PI is crucial for accessing appropriate treatment and significantly improving patient outcomes.

Material and Methods: Prospective analysis and clinical assessment were accomplished for 1591 affected individuals with

PI through the Jeffrey Modell Foundation (JMF) program. Molecular diagnoses were performed using a next-generation sequencing approach by analyzing a 575 PI-related gene panel.

Results: Overall, 21% (334/1591) of the patients studied were genetically characterized. The genetic analysis revealed 5 cases with heterozygous variants in the ELANE gene (4 males and 1 female; median age 2.75 years), from Latin America (n = 3), Asia (n = 1), and Europe (n = 1). All diagnosed cases presented a similar clinical manifestation, starting in the first months of life and characterized mainly by neutropenia (in all 5 patients), abnormality of granulocytes (n = 3), recurrent infections (n = 2), fever (n = 2), and anemia (n = 2). All identified disease-causing variants, 4 missense and 1 nonsense variant in the last exon, were located in the Peptidase S1 domain and expected to have a dominant-negative effect.

Conclusion: JMF emphasizes the critical importance of early and accurate PI diagnosis for timely access to effective treatments and improvement of patient outcomes, as exemplified by the management of ELANE-related neutropenia through early identification of disease-causing variants and Granulocyte-Colony Stimulating Factor (G-CSF) therapy.

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P09.008.B Combining next generation sequencing and cytogenetics for the diagnosis of ICF1 syndrome

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Background: Immunodeficiency, centromeric instability, and facial dysmorphism (ICF) syndrome type 1 (ICF1, OMIM #242860) is a rare autosomal recessive disorder characterized by immunodeficiency of variable extent, mild facial anomalies and chromosomal instability. It is caused by variants in the *DNMT3B* gene.

Material and Methods: NGS, G-band cytogenetic analysis.

Results: The patient was a 1-year-old boy who presented with recurrent infections, hypogammaglobulinemia and low B cells, pointing to a primary immunodeficiency (PID). Genetic analysis using targeted NGS-PID panel (461 genes), identified two rare heterozygous variants in the *DNMT3B* gene. The first variant, c.1957G>A/p.Asp653Asn had been described in the literature in a patient with ICF1 syndrome in *trans* with another likely pathogenic variant and was therefore classified as likely pathogenic (LP). The second variant, c.2185G>A/p.(Ala729Thr), considered pathogenic by most computational predictors, had not been previously reported in the literature and was classified as a variant of uncertain significance (VUS). Parental testing confirmed that the variants were in *trans* in the patient. Given the presence of a LP and VUS in the patient, we performed cytogenetics analysis to confirm ICF1. Chromosomal analysis showed that 44% of metaphases displayed alterations in the juxtacentromeric heterochromatin of chromosomes 1 and 16, including multibranched configurations and heterochromatin decondensation, findings characteristic of ICF1 syndrome.

Conclusion: The cytogenetic findings allowed to reclassify the c.2185G>A/p.(Ala729Thr) variant as LP, confirming the diagnosis of ICF1 syndrome in the patient. This study illustrates the importance of integrating modern sequencing techniques (NGS) and classical cytogenetic methods to reach a definitive genetic diagnosis.

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P09.009.C A novel mutation in proteasomal gene *PSMD7* results in dysregulated protein degradation, enhanced inflammasome activation, and interferon response

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Background: Necrotizing fasciitis (NF), is a severe infectious disease affecting soft tissues. Recent findings suggest that germ line mutations of genes regulating the innate immune responses may predispose to NF and trigger an autoinflammatory form of NF. We describe a patient who presented with NF and carried heterozygous variants in *TRIM23*, *PSMD7* and *PSMA5*. PSMD7 is a proteasome 26S subunit, which is critical for degradation of ubiquitinated proteins in the proteasome. Increased *PSMD7* expression has been associated with cancer, but the impact of PSMD7 in inflammation is not well understood. The patient had persistent severe inflammation which was ameliorated with IL-1β receptor antagonist anakinra.

Material and Methods: To characterize the role of PSMD7 in immune cells, we utilized CRISPR-Cas9 to create THP-1 cell lines carrying heterozygous *PSMD7* knockout (KO). Patient-derived macrophages and *PSMD7 KO* cells were stimulated with lipopolysaccharide (LPS) and inflammasome activators. NLRP3 inflammasome activation, proteasome function and interferon response were studied.

Results: Patient derived macrophages and *PSMD7 KO* cells responded to NLRP3 inflammasome activation with increased IL-1β secretion, and expression of interferon beta 1, followed by enhanced interferon response. Loss of *PSDM7* affected proteasomal activity, with patients and KO cells exhibiting higher intracellular and extracellular ubiquitin levels.

Conclusion: Ubiquitination is essential for maintaining homeostasis under steady state and inflammatory conditions, especially in regulation and degradation of NLRP3 inflammasome. Loss of proteasomal lid subunit PSMD7, elevates ubiquitin levels, enhances NLRP3 inflammasome activation and increases interferon response. Proteasomal dysfunction may predispose to hyperinflammation and NF.

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P09.010.D Exome sequencing to unravel monogenic autoinflammatory disorders in Indian population: A preliminary experience

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Background: Autoinflammatory disorders (AIDs) are genetically heterogeneous conditions characterized by episodic or persistent unprovoked inflammation caused by aberrations in various components of innate immunity. The clinical diagnosis of these disorders is challenging due to their highly diverse and overlapping phenotypes.

Material and Methods: We recruited 193 families (200 individuals) with clinical findings suggestive of AIDs from September 2021 till August 2023. Exome sequencing (ES) was carried out after detailed clinical evaluation.

Results: The predominant clinical findings at presentation in 200 affected individuals were recurrent fever (82%), arthritis (60%), urticaria (44%), abdominal pain (21%), oral ulcer (21%), uveitis (14%), and cutaneous polyarteritis nodosa (8%). Most of the patients exhibited elevated levels of inflammatory markers (70%). Molecular diagnosis was achieved in 58 out of 193 families (30%). Of these, twelve (20%) families had type I interferonopathies (*DNASE1L3, C1QA, C1QB, ACP5, DNASE2, ADA2*), seven (12%) families had defects affecting inflammasomes (*MVK, NLRP12, NLRP3*), and sixteen (28%) families had non-inflammasome related conditions (*NOD2, TNFRSF1A, LPIN2, SLC29A3, TNFAIP3, RIPK1, DOCK11, IL1RN*). Twenty-three (40%) families had variants in genes causing other monogenic conditions mimicking AIDs (*LEMD3, AP1G1, TBXAS1, LACC1, ELANE, TGFB1, PLG, GPNMB, KRAS, NCSTN, RETREG1, TBXAS1, STXBP2, IRAK4, NFKBIA, JAK3, PRG4*). We identified 50 disease-causing variants in 34 distinct genes in 58 families. Among these, twenty-one (42%) variants were novel.

Conclusion: We highlight the utility of ES as a diagnostic tool in identification of monogenic AIDs, which will aid in ending the diagnostic odyssey and facilitate in personalized therapy.

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P09.011.A Systematic assessment of exome data for copy number variants followed by independent quantitative assays reveals a high rate of CNVs affecting immune genes

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Background: The etiology of immunodeficiencies with suspected Mendelian inheritance is comparatively poorly understood. In an attempt to identify novel genes underlying these phenotypes, we systematically analyzed exome data for CNVs beyond established disease genes.

Material and Methods: Exome sequencing was performed for 112 cases with suspected immunodeficiency (46% singletons, 47% trios), rare CNVs determined using ExomeDepth. In a first tier, a diagnostic panel of 449 genes was analyzed (PMID: 35748970). In 90 patients, the analysis was extended to all genes and confirmed with customized MLPA assays in a subset of cases.

Results: We identified a pathogenic SNVs (LP/P) in seven established genes (20% diagnostic yield). Systematic analysis of exome data revealed 1.4 CNV per individual, in total 112 CNVs affecting 156 genes, including 69 immune-related genes. MLPA-based validation of the first 44 CNVs (22 duplications, 22 deletions) confirmed 29 CNVs (66%) including all inherited ones, although the size of seven differed. As expected, confirmed CNVs were significantly larger ($p = 0.0047$; median: 4 exons, range 1–23) than unconfirmed ones (2 exons (1–4)). Nevertheless, 52% of the smaller CNVs (1–4 exons) could be confirmed. These were good candidate genes, as they encode proteins of metabolite interconversion enzymes, RNA metabolism and protein modifying enzymes.

Conclusion: Genome-wide CNV-analysis from exome data allowed detecting novel candidate genes for primary immunodeficiencies, while whole genome sequencing is advantageous in molecular characterization. Heterogeneity is high, since mostly a single case each was identified, requiring multicentric cooperation for genetic validation. Further studies will allow a better understanding of primary immunodeficiencies.

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P09.012.B Building an immune cell atlas from diverse Latin American (LATAM) populations

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Latin American (LATAM) populations are under-represented in genetic and genomic studies, hampering the discovery and translation of such studies across populations. We aim to expand our understanding of these populations by mapping the impact ancestral genetic diversity has on cell composition and gene regulation in peripheral blood mononuclear cells (PBMC) in healthy individuals using single-cell technologies. Here, we describe a new experimental workflow to enrich common and rare immune cell types for single-cell (sc) multiomic assays.

PBMCs are collected and cryopreserved in Mexico, Argentina, Colombia, Peru, Chile and Uruguay. We applied spectral flow cytometry (FACS) to enrich 13 different immune cell types into six buckets: CD4 T, CD8 T, B, NK, myeloid (monocytes and dendritic cells) and rare (Treg, gdT, NKT, ILC, progenitor B, dnT and dpT cells). We generated scRNA-seq and CITE-seq (130 cell surface proteins) data.

We developed a 16-colour spectral FACS panel to identify key cell type markers for sorting. Post-sort purity checks reveal minimal cellular contamination in each sorted bucket. Our initial scRNA-seq and CITE-seq data set demonstrates the sorting strategy provides better cell capture; increasing detection of rare cell types, such as Treg, which increase in proportion by ~3-fold in scRNAseq data compared to FACS.

Here, we demonstrate our approach enables robust data generation and we will scale this up by generating scRNA-seq, CITE-seq and scATAC-seq data for 160 individuals, capturing ~2M cells. This comprehensive immune cell atlas will improve our understanding of genetic and environmental influences on the immune system from diverse LATAM populations.

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P09.013.C Type I interferon response in MSMD patients: evaluation of IFN signature score as a biomarker

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Background: Mendelian Susceptibility to Mycobacterial Diseases (MSMD) is a rare immunodeficiency that increases susceptibility to infections from pathogens like BCG vaccine, Mycobacterium tuberculosis, non-tuberculous species, and Salmonella strains. The role of IFN-I in MSMD is not fully understood, and its effects on risk stratification and treatment options are not well understood. This study examines the regulation of interferon-stimulated genes in MSMD patients to understand their impact on disease progression and IFN signature test scores.

Material and Methods: The study involved 18 MSMD cases and 20 healthy controls, using RNA isolation, cDNA synthesis, and SYBR-Green real-time quantitative PCR methods to determine gene expression levels involved in the Type I IFN pathway including STAT1, PRKR, IFI27, IFI44, IFI44L, IFIT1, RSAD2, MX1, and LY6E. Statistical analyses were performed using SPSS 16.0 software, with a p-value of <0.05 considered significant.

Results: In 77% of MSMD patients, a high IFN signature score (>7) was observed, attributed to increased expression of the IFIT1, PRKR, and MX1 genes compared to controls ($p = 0.004$, $p = 0.03$, and $p = 0.02$). Conversely, the expression levels of the IFI27 and RSAD2 genes were found to be decreased ($p = 0.01$ and $p = 0.02$).

Conclusion: The study evaluated the activation of the Type I interferon (IFN-I) pathway in MSMD patients and explored the use of the IFN signature test as a biomarker. The results suggest that IFN-I inhibitors could be a potential treatment option for patients with pronounced IFN-I activation, in addition to standard treatments like antibiotics, IFN γ therapy, or hematopoietic stem cell transplantation.

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P09.014.D Blended phenotypes in congenital neutropenia: Contribution of consanguinity

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Background: Congenital neutropenia (CN) is a rare disorder (1/10,000) characterized by reduced neutrophil counts and recurrent infections. CN can be isolated or syndromic, and is linked to >30 genes. Traditionally, CN with additional clinical features has been linked to monogenic causes. However, considering the emerging concept of blended phenotypes, we investigated whether multiple pathogenic variants in distinct genes could explain CN with extra-clinical findings.

Material and Methods: We evaluated 24 CN patients without pathogenic variants in *ELANE*, *HAX1*, *TAZ*, *SLC37A4*, or *SBDS*. Nine had additional unexplained clinical findings. Clinical and/or whole exome sequencing (Twist 2.0, KAPA HyperCap) was performed for all. Microarray was conducted in 19 individuals to assess CNVs and homozygosity regions.

Results: Eight (33.4%) of 24 individuals had at least one monogenic variant explaining CN. Six had multiple pathogenic variants in at least two genes, explaining their complex phenotype. Interestingly, an individual with blended phenotype diagnosed with benign ethnic neutropenia due to a nonrare variant in *ACKR1*, congenital hearing loss and abdominal pain attacks explained by variants in *GJB2* and *PPOX*. Notably, five of six individuals with reported parental consanguinity or extensive homozygosity regions had blended phenotypes. One consanguineous individual had a homozygous *RINT1* variant, explaining both liver failure and neutropenia.

Conclusion: This study highlights the complicated genetic landscape of CN in a consanguineous population. Additional phenotypic features in individuals with consanguineous parents is a good indicator of multiple disease-causing genes in our cohort. This emphasizes the need for comprehensive genetic testing to fully explain their clinical phenotype.

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P09.015.A Thymus-regulating genetic variant rs2204985 associated with overall survival in stem cell but not in solid organ transplantation

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Background: The thymus is crucial for educating T cells to achieve central immunological tolerance, a key feature in successful transplantation. Recently, variants in the T cell receptor (TCR) gene were linked to thymus function [1]. We and others have reported inferior overall survival (OS) with donor rs2204985 AA genotype in allogeneic stem cell transplantations (HSCT) [2-3].

Material and Methods: We investigated four TCR variants in (i) HSCT cohorts from Finland (n = 765 donors, n = 1305 recipients), UK (n = 334 donors, n = 403 recipients), and Poland (n = 102 donors, n = 168 recipients), (ii) an independent HSCT cohort from Germany (n = 703 donors, n = 1331 recipients); and (iii) two solid organ transplantation cohorts from Finland (n = 1025 kidney transplants [4]; n = 666 liver transplants [5]). Data were analyzed using meta-analysis of multivariate cox proportional hazard survival models against OS.

Results: In a meta-analysis of HSCT cohorts (i) and (ii), the rs2204985 G allele was additively associated with improved OS (HR = 0.880, 95% CI 0.777–0.996, p-value 0.043), supporting our previous meta-analysis results (AA genotype HR = 1.23, 95% CI 0.94–1.96) [3]. No significant associations were observed in solid organ transplantations.

Conclusion: The T cell receptor variant rs2204985, which regulates thymus function, is associated with HSCT outcome. This finding is functionally plausible, as the entire hematopoietic system is replaced in HSCT, requiring donor immune cells to undergo thymic education and selection. In solid organ transplantations, peripheral tolerance may be more important.

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P09.016.B Transcripts containing retrotransposable elements play a regulatory role in modulating the functions of tumor infiltrating lymphocytes

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Background: Unravelling Tumor Infiltrating Lymphocytes (TIL) heterogeneity is essential to develop more effective

immunotherapies. We have recently highlighted the role of LINE1, the predominant class of Retrotransposable Elements (RE), in regulating TIL exhaustion by integrating in transcripts related to T cell activation and downregulating their canonical isoforms. Nevertheless, the role of RE expression as a determinant of TIL diversity and function remains poorly explored.

Material and Methods: We have isolated CD3+ and CD45+ T cells from tumor resections of Non-Small Cell Lung Cancer (NSCLC) and Colorectal Cancer (CRC) patients. Due to the challenging task of studying transcripts containing RE with current single-cell technologies, we performed both long- and short-read scRNA-seq. We have developed a pipeline to analyze long-read data to generate a cancer-specific Atlas of RE-transcripts that we quantified at isoform level using full-length short-read data.

Results: We described approximately 40,000 novel transcripts that are specific to NSCLC and CRC, with some shared instances. Almost 46% of them included REs, resembling their genomic distribution. Trajectory inference analysis confirmed the positive correlation between LINE1 and exhausted TILs, but it also revealed an enrichment of Alu-containing transcripts in active T cells, while regulatory T cells (Treg) and follicular T helper cells (Tfh) mostly expressed transcripts containing the LTR class.

Conclusion: To elucidate the relevance of RE-transcripts in TIL identity, we will leverage CRISPR-Cas13 system to specifically modulate them and evaluate their impact, introducing the innovative concept that RE transcripts may serve as novel immunological checkpoints and a promising avenue for cancer therapy.

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P09.017.C Monoallelic variants in *PSMB8* cause an autoinflammatory syndrome with immunodeficiency through a dominant-negative mechanism

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Introduction: Proteasome-associated autoinflammatory syndromes (PRAAS) are disorders caused by immunoproteasome dysfunction. The immunoproteasome is a large protein complex, its core consisting of the catalytic subunits β1i, β2i and β5i. We describe two cases with monoallelic de novo variants in *PSMB8*, encoding β5i, showing features distinct from the classical PRAAS phenotype associated with autosomal recessive mutations.

Materials: Two independent individuals were identified through genome sequencing. Subsequently, phenotypic data, immunophenotyping profiles and interferon scores were collected. Variant effects on proteasome assembly were assessed by complexome profiling—a mass spectrometry-based proteomics technique—on interferon-gamma-stimulated patient-derived fibroblasts.

Results: Individuals presented with early-onset autoinflammation with immunodeficiency. Features consistent with PRAAS included systemic inflammation, microcytic anemia, skin rash, myositis and liver dysfunction, although lipodystrophy nor joint contractures were present. Patients also presented recurrent infections, chronic inflammatory airway disease and pulmonary hypertension, and enteropathy. Immunological investigations in both patients revealed B and NK lymphopenia, reduced switched memory B cells with hypogammaglobulinemia, and an elevated interferon signature. Genome sequencing identified a de novo variant in *PSMB8* in each individual: NM_148919.4:c.704C>A;p.(Ala235Asp) and NM_148919.4:c.269C>T;p.(Ser90Phe). Complexome profiling of p.(Ala235Asp) revealed disrupted immunoproteasome assembly, resulting in reduced abundance while leaving the constitutive proteasome unaffected. Furthermore, there was an accumulation of assembly intermediates of the immunoproteasome complex that did not contain β5i.

Conclusion: We describe two individuals with early-onset systemic autoinflammation and immunodeficiency, caused by monoallelic de novo variants in *PSMB8* that disrupt immunoproteasome assembly, likely acting through negative dominance. This report expands the spectrum of PSMB8-associated disease and PRAAS in general.

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P09.018.D Molecular features of Familial Mediterranean Fever and Pro-inflammatory Cytokines Levels in Patients with Various *MEFV* Pathogenic Variants

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Background: Familial Mediterranean fever (FMF) is an autoinflammatory disease manifested by recurrent fever with signs of systemic inflammation caused by *MEFV* pathogenic variants that lead to inflammasome hyperactivation and hyperproduction of IL-1β and IL-18. FMF is a disorder with variable expressivity and penetrance: heterozygous variants carriers can develop FMF phenotypes.

Material and Methods: The study included 1271 samples positive for pathogenic variants in *MEFV*, identified by sequencing of 2, 3, 5, 10 exons. Concentrations of CRP, IL-6, IL-8 and IL-18 were measured by ELISA for 3 groups: 95 patients (FMF) with different MEFV genotypes (homozygous (n = 18), compound-heterozygous (n = 27), heterozygous (n = 34), heterozygous variants with

variant of unknown significance (VOUS) (n = 9) and only VOUS (n = 9)), patients with systemic lupus erythematosus (SLE, n = 49) and a group of healthy donors (HD, n = 16).

Results: From 1271 samples, homozygous for pathogenic variants was 11,49%, compound-heterozygous - 27,69%, heterozygous - 42,17%, heterozygous/VOUS - 5,90% and VOUS - 12,75%. 32 variants were detected, the most common were p.Met694Val (39,89%), p.Val726Ala (19,12%), p.Met680Ile (13,86%). Concentrations of CRP, IL-6 and IL-18 in FMF group was significantly higher than in HD and SLE groups (P < 0.0001). Concentration of IL-18 was significantly higher in all FMF subgroups than in HD. Comparing IL-18 levels between different pathogenic *MEFV* variants, samples with «aggressive» variants (p.Met694Val and p.Met680Ile) were characterized by increased concentration of IL-18 (P = 0.0411).

Conclusion: Patients with FMF in this study characterized by classic spectrum of *MEFV* variants with high prevalence of «aggressive» genotypes. Pro-inflammatory markers' levels depend on *MEFV* genotype and specific variants.

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P09.019.A Clonal Hematopoiesis in People Living With HIV: Association With Aging and Inflammation

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Background: Clonal Hematopoiesis of Indeterminate Potential (CHIP) is the accumulation of leukemogenic somatic mutations, strongly linked to aging, increased mortality, and age-related diseases. One proposed mechanism is that CHIP marks chronic inflammation. If so, People Living With HIV (PLWH), who already experience heightened inflammation, should (i) exhibit accelerated aging, (ii) have increased CHIP prevalence, and (iii) show even greater inflammation and more severe age-related phenotypes when CHIP is present. Since (i) and (ii) are well established, we focus on (iii)—how CHIP and HIV are linked to inflammation and aging.

Material and Methods: We screened 1,636 participants (≥60 years) from the Swiss HIV Cohort Study (SHCS) for CHIP with a targeted panel covering 24 genes, with a median sequencing coverage of 737X. CHIP was detected in 414 individuals (25.3%).

Results: As expected, CHIP was associated with age, accelerated epigenetic aging, and increased all-cause mortality, with stronger associations for larger clone sizes and multiple CHIP variants. However, age-related diseases demonstrated either the absence of association with CHIP, or gene-specificity.Additionally, CHIP was associated with lower CD4-T-cell count, higher CD8-T-cell count, lower CD4:CD8 ratio, and higher HIV load, even after adjusting for ART initiation, potentially suggesting an increased inflammation level and immune dysregulation.

Conclusion: We demonstrated expected associations of CHIP with a proxy of inflammation and aging in PLWH, but not all age-related diseases showed consistent trends, which might mean a complex interaction of HIV and CHIP and different CHIP-related effects on health in the context of HIV.

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V. Timonina: None. **K. Popadin:** None. **J. Fellay:** None.

P09.020.B Germline variants and somatic mosaic chromosomal alterations affect COVID-19 vaccine immunogenicity, infectious diseases, and autoimmune diseases

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Background: Vaccine immunogenicity is influenced by the vaccinee's genetic background.

Material and Methods: We performed GWAS of vaccine-induced SARS-CoV-2-specific IgG antibody titers and T-cell immune responses in Japanese cohorts (mRNA-1273 and BNT162b2, *N* = 2086).

Results: We identify associations with SARS-CoV-2-specific antibody titers at the immunoglobulin heavy chain (IGH) and MHC locus, and associations with the T-cell responses at MHC. The lead variants at IGH contain a missense variant (rs1043109-C; p.Leu192Val) in the Immunoglobulin Heavy Constant Gamma 1 gene (*IGHG1*), which strongly decreases the antibody titers ($\beta = -0.54$) and is rarely present in Europeans, highlighting the importance of genetic studies in various populations. SARS-CoV-2-specific antibody serostatus data from the UK Biobank (UKB; *N* = 152,906) independently reveal associations at the IGH, MHC, immunoglobulin kappa light chain and 16p11 locus. HLA imputation analyses in the Japanese cohorts pinpoint *HLA-DRB1* as the strongest determinant of the antibody titers, and *HLA-DRB1* and *HLA-DPB1* as that of the T-cell responses within MHC. Blood proteome profiles (2,932 Olink Explore proteins) from 4,236 individuals demonstrate that antibody titer-associated variants modulate circulating immune regulators, including LILRB4 and FCRL6. In addition to germline mutations, age-related somatically expanded mosaic chromosomal alterations (mCA) affecting MHC and IGH impair SARS-CoV-2-specific antibody production. Furthermore, MHC/IGH-affecting expanded mCAs confer risk of infectious and immune diseases, such as sepsis and Graves' disease, in Japanese and European biobanks ($N_{Total} = 663{,}133$). Expanded mosaic loss of sex chromosomes (X or Y) impact such phenotypes including rheumatoid arthritis.

Conclusion: Our results underscore the contribution of both germline and somatic mutations to adaptive immunity functions.

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P09.021.C A genomic upgrade to 2139 genetic variants in the interactive coagulation factor IX database provides enhanced insights into hemophilia B

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Background: *F9* gene variants are frequently associated with the rare bleeding disorder hemophilia B. These have been compiled in our widely-used coagulation factor IX (FIX) variant database (https://www.factorix.org) from 2013 and updated in 2022. Here,

we report the introduction of new variant analysis tools, along with corrections and a significant increase of new *F9* variants.

Material and Methods: A total of 513 new *F9* variants and 2358 new clinical cases were analyzed, resulting in an updated count of 2139 *F9* variants and 7710 related patient cases. *F9* variants were identified from peer-reviewed publications and genomic databases, and incorporated into our database. Given a recent increase in reported pre-mRNA alterations in *F9*, we implemented deep neural network-based tools and phenotypical predictions in our dataset.

Results: The *F9* variants cover 424 (92%) of the 461 FIX residues, including 20 newly reported ones. These total 1542 point mutations, 336 deletions, 97 polymorphisms, 69 duplications, and 95 other types. While point variants remain predominant (72%), non-coding sequence variants increased by 119%, with 361 point variants in UTRs or introns. According to patient cases, p.Arg43Trp and p.Ile443Thr showed severity changes. Inhibitor risk in severe hemophilia B cases was calculated as 6.4%.

Conclusion: New recent expansions to the FIX database and its biocuration process have been described. Most variants are associated with minor structural changes in the FIX protein, while others are associated with alterations in pre-mRNA processing of the gene. The updated database will assist clinicians and researchers in assessing treatments for hemophilia B patients.

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P09.022.D Mainstreaming genomic testing for paediatric inborn errors of immunity

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Background: A mainstreaming model-of-care (MoC) was established in Queensland, Australia to support genomic testing (GT) for paediatric inborn errors of immunity (IEI). The model's feasibility and acceptability among clinicians and families have previously been demonstrated. However, its long-term impact remains unknown, and this clinical cohort has not yet been characterised within an Australian context. This study aimed to i) evaluate the long-term impact of the MoC on GT practices and patient outcomes ii) describe the first Australian cohort of paediatric IEI

Methods: A retrospective audit and chart review were conducted to identify all instances of paediatric IEI GT in Queensland from 2017–2024. Data collected included demographics, indication for GT, GT instances, testing modality, results, and testing outcomes. GT ordering and interpretation behaviours were reviewed to evaluate the impact of the MoC.

Results: 261 patients were identified as meeting the inclusion criteria for the audit. The mean age of GT was seven years, and the most common indication for testing was inflammatory bowel disease (n = 25, 14%). There was a linear increase in the number of patients tested from 2017–2018 (n = 40) to 2021 (n = 50), which reduced post-MoC implementation (n = 36 2022; n = 37 2023; n = 26 2024). Diagnostic rates improved post-MoC implementation, at 18% and 23%, respectively. Improved documentation related to GT was noted, including recording informed consent, results, variant interpretation, and familial considerations.

Conclusion: A mainstreaming MoC improves paediatric IEI GT practices. Developing an IEI database will allow for ongoing tracking of GT, description of clinical cohort, and evaluation of future services.

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P09.023.A Genome-wide aggregated *trans*-effects analysis detects key role of immune checkpoint signalling in Sjögren's disease

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Background: Sjögren's disease (SjD) is an autoimmune condition that frequently co-occurs with rheumatoid arthritis. We have previously reported that genome-wide aggregated *trans*-effects (GATE) analysis identifies up to 15 core genes for rheumatoid arthritis, of which seven encode immune checkpoints. The objective of this new study was to determine whether these core genes are relevant to SjD also.

Material and Methods: Using publicly available summary statistics for genotype associations with transcript levels in whole blood (eQTLGen) and circulating proteins on the Somalogic or Olink platforms (DeCODE, UK Biobank), GATE scores were calculated in 950 cases of primary SjD (without rheumatoid arthritis when first diagnosed) and 459105 non-cases of European ancestry in UK Biobank.

Results: The top core gene for rheumatoid arthritis - *PDCD1*, encoding the immune checkpoint PD-1 - was also the top core gene for primary SjD (standardised GATE score log odds ratio 0.23, $p = 2 \times 10^{-7}$). Of the six other immune checkpoint genes identified as core genes for rheumatoid arthritis, three - *TIGIT*, *TNFRSF14*, *LILRA4* - were associated in the same direction with SjD at $p < 0.01$.

Conclusion: These results establish that genetic downregulation of immune checkpoint signalling increases risk of both SjD and rheumatoid arthritis. The causal role of *PDCD1* is supported by reports of SjD developing as a rare adverse effect of PD-1 inhibitors used to treat cancer. As no disease-modifying treatment for SjD is available, one implication is that PD-1 agonists, now at clinical stage, should be trialled for SjD.

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P09.024.B Characterization of a novel *FLI1* mutation in a family with thrombocytopenia and other congenital malformations

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Background: FLI1-related thrombocytopenia (FLI1-RT) is an extremely rare disease caused by mutations in *FLI1*, encoding a transcription factor which mutations cause a non-syndromic thrombocytopenia with enlarged platelets. In a family with

thrombocytopenia associated with congenital anomalies, we identified a novel, heterozygous single nucleotide deletion of *FLI1*, which has been characterized from a molecular point of view.

Material and Methods: Genetic analyses were performed by Whole Exome Sequencing and CGH array. Platelet morphology was assessed by light microscopy and transmission electron microscopy. In vitro studies were performed in HEK293T and HeLa cells after overexpression of wild-type or mutant *FLI1* and analysed by immunoblotting, immunofluorescence and dual luciferase assays. In vivo platelets were analysed by immunoblotting and flow cytometry.

Results: The mutant frameshift protein is unexpectedly highy overexpressed in patients' platelets and in vitro models due to its increased stability. Although localised in the nucleus, the mutant protein loses its transcriptional activity, supporting its pathogenic role. Consistently, patients' platelets show alterations of the expression of several biomarkers directly associated with FLI1-RT. The two affected individuals have haematological features consistent with FLI1-RT, such as moderate thrombocytopenia, large platelet size with giant alpha granules and reduced delta granules content.

Conclusion: For the first time, FLI1-RT is observed with other extra-haematological abnormalities, such as cardiac defects, upper limb anomalies and polymicrogyria. Despite extensive genetic analysis, we have not identified other co-segregating mutations associated with the extra-haematological phenotype, suggesting that specific mutations of FLI1 may be responsible for the aetiopathogenesis of a more complex phenotype.

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P09.025.C Exploring ERVL-MaLR dynamics in tumor-infiltrating tregs to unveil novel immunotherapeutic candidates

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Background: The tumor microenvironment (TME) is characterized by heterogeneous immune infiltration, where the dynamic interaction between effector T cells and regulatory T cells (Tregs) plays a central role in driving cancer progression and influencing patient survival. Recently, Retrotransposable Elements (REs) have emerged as key regulators of tumor-infiltrating lymphocyte (TIL) function. We have demonstrated that LINE1-derived transcripts impair the activity of exhausted TILs, but also represent targets to restore T cell effector function. Despite this, the role of RE expression in shaping the function and behavior of pro-tumoral Tregs remains largely uninvestigated.

Material and Methods: REs expression in TILs from tumor and adjacent normal tissues across six independent datasets was quantified using IRescue, a tool developed in our lab for analyzing 10X single-cell RNA sequencing data. Chromatin accessibility of REs in TILs was further investigated using single-cell ATAC sequencing data. Binding motif analysis was performed to identify transcription factor regulatory networks associated with RE activity.

Results: Among all RE families, ERVL-MaLR expression was consistently associated with Tregs across all tumors analyzed. Specific ERVL-MaLR subfamilies were found to be upregulated in tumoral Tregs compared to their counterparts in adjacent normal tissues. Binding motif analysis of ERVL-MaLR-accessible regions identified BATF as a potential transcription factor involved in the regulation of their expression.

Conclusion: This analysis reveals a previously uncharacterized association between ERVL-MaLR expression and tumoral Tregs. Deciphering the regulatory mechanisms underlying ERVL-MaLR expression holds potential for advancing Treg-targeted immunotherapy approaches.

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P09.026.D Demonstration of Microchimerism by Short Tandem Repeat Analysis in a Patient with Homozygous Exons 1–3 Deletion in *DCLRE1C* Gene

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Background: Severe combined immunodeficiency (SCID) is a group of inherited disorders characterised by severe dysfunctions of the immune system. Transplacental maternal engraftment (TME), the presence of maternal T cells in peripheral blood/bone marrow prior to transplantation, has been reported in patients with SCID. TME is mostly asymptomatic, rarely causing Graft-Versus-Host disease.

Material and Methods: A 6-month-old girl whose lymphocyte profile analysis was compatible with SCID was evaluated. Short tandem repeat (STR) analysis was performed in the skin biopsy of patient and peripheral blood of patient and maternal peripheral blood. Whole exome sequencing was performed from DNA obtained from the skin biopsy of patient.

Results: In STR analysis of patient's peripheral blood and skin biopsy, peaks belonging to maternal STR loci were observed. STR analysis data obtained from maternal and patient's peripheral blood were evaluated as compatible with TME. Short tandem repeat analysis of patient's skin biopsy showed low peak height peaks of maternal STR loci supporting Graft-Versus-Host disease. Whole exome sequencing analysis identified approximately 28 kb homozygous deletion encompassing exons 1–3 of *DCLRE1C* gene. *DCLRE1C* gene has been associated with 'SCID, Athabascan type' and 'Omen Syndrome' with autosomal recessive inheritance.

Conclusion: The ability to assess the rate of TME in patients with SCID will depend on the severity of Graft-Versus-Host disease. TME may pose difficulties in donor selection for haematopoietic stem cell transplantation. summary, this study demonstrates the molecular genetic challenges due to Graft-Versus-Host disease and microchimerism and importance of considering microchimerism in whole exome analysis in SCID patients.

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L. Yilmaz Celik: None. **T. Esen:** None. **C. Aydogmus:** None.

P09.027.A Advancing diagnosis of inborn errors of immunity through exome reanalysis for novel gene-disease associations

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Background: inborn errors of immunity (IEI) are a group of monogenic conditions presenting with immunodeficiency,

autoimmunity and/or autoinflammation. The International Union of Immunological Societies standardizes gene-disease associations, with the recently published 2024 update including over 60 novel entities comparing with the 2022 nosology. We analyzed the database from a genetic diagnostic laboratory with interest in IEIs for variants underlying these novel conditions.

Material and Methods: we reviewed 2082 exome-based NGS studies for variants in 63 novel IEI genes. We excluded frequent variants (MAF>1%), intronic variants with SpliceAI score <0.2, variants with coverage <10x, or with a VUS-low classification.

Results: we identified seven cases with eight candidate heterozygous variants for AD conditions. Two cases had a likely pathogenic variant: a missense *FOXI3* variant in a patient with Alagille syndrome and no immunodeficiency; and a compatible nonsense *OTULIN* variant in a patient with recurrent mild bacterial infections. Two cases had VUS-high variants: one case with a severe hyperthermia reaction and a *JAK1* (VAF 42%) and *ERN1* variant, associated with autoinflammation and immune dysregulation, respectively; one case with recurrent fever and a *JAK1* (VAF 12%) variant. Finally, three cases had one VUS-mid: one case with IgA glomerulonephritis and *ERN1* variant; one case with IgG3 deficiency and recurrent pneumonitis and an *ALPK1* variant, associated with autoinflammation; and one case with *NLRP12*-associated autoinflammation and a *PLCG1* variant, associated with immune dysregulation.

Conclusion: the rapid discovery of IEI genes should lead to reanalysis of exome-based tests, as underlying diagnoses can be detected, improving clinical care.

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J.D. Da Silva: None. **A.R. Soares:** None. **I. Alonso:** None.

P09.028.B Genetic diagnosis of hereditary autoinflammatory syndromes in patients with fever of unknown origin

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Background: Fever of unknown origin (FUO) is a challenging clinical entity, characterized by unexplained repetitive elevation of temperature ≥38.3. The etiological spectrum of FUO is wide. Hereditary autoinflammatory syndromes (HAS) may be one of the main cause of FUO. The aim of this work is to determine the diagnostic value of genetic testing of patients with FUO.

Material and Methods: 442 patients with FUO were selected. The coding sequences of the IL10, IL10RA, IL10RB, IL1RN, LPIN2, MEFV, MVK, NLRP3, NOD2, PLCG2, PSTPIP1, TNFRSF1A genes were tested with NGS panel (ParseqLab©, Russia).

Results: At least one variant in the studied genes was detected in 149 (33.7%) cases. In total 201 variants were found. 132 (65.7%) of them were variants with unknown significance (VUS). *NOD2* gene had the largest number of VUS (52 variants). Likely pathogenic (N = 40, 19.9%) and pathogenic variants N = 29, 14.4%) were found in *MEFV* (N = 64), *MVK* (N = 2) and *NLRP3* (N = 2) genes. Among all 442 patients, diagnosis was verified for 25 individuals (5.7%): 22 patients had Familial Mediterranean fever (*MEFV* gene) and 3 had Muckle-Wells syndrome (*NLRP3* gene). Also, 2 patients were carriers of *MVK* pathogenic variants. 103 patients (23.3%) had genotype with unknow significance (VUS for diseases with AD inheritance) and 21 (4.8%) had genotype with no clinical significance (VUS for diseases with AR inheritance). Negative results of genetic testing were obtained for 293 patients (66.3%).

Conclusion: Prevalence of genetically confirmed HAS in patients with FOU was 5.7%. Group of FOU patients characterized by high prevalence of VUS (65.7%).

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P09.029.C Genetic susceptibility to idiopathic pulmonary fibrosis: insights from Sardinian patients

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Background: Idiopathic pulmonary fibrosis (IPF) is a severe interstitial lung disease marked by progressive scarring, loss of lung function, and, ultimately, respiratory failure. Although environmental factors play a role, the growing recognition of genetic predisposition is reshaping our understanding of disease mechanisms. With its distinct genetic background, the Sardinian population provides a unique opportunity to unravel the interplay between common and rare genetic variants in IPF pathogenesis and progression.

Material and Methods: This study leveraged the Sardinian genetic landscape to investigate both the impact of *HLA* alleles and haplotypes and the contribution of rare genetic variants to IPF susceptibility and progression. Through genotyping and WES in a well-characterised cohort, we explored allele frequencies, haplotypes, and pathogenic variants, correlating these findings with clinical outcomes, including response to antifibrotic therapies.

Results: The *HLA-DRB1*04:05* allele and a extended Sardinian haplotype (*HLA-A*30:02, B*18:01, C*05:01, DQA1*05:01, DQB1*02:01, DRB1*03:01*) were strongly associated with slower disease progression, improved survival (Log Rank = 0.032 and 0.01), and better pulmonary function at 36 months ((DLCO%p; p = 0.005 and 0.02). Rare pathogenic variants in *TERT, RTEL1, PARN*, and *CTC1* were identified in 22% of patients, with 12% affecting genes beyond the telomerase pathway, hinting at novel disease mechanisms. Preliminary findings suggest that TERT variants may influence

Conclusion: These unique and original findings provide an unparalleled lens to decode IPF pathogenesis. The Sardinian genetic landscape unveils novel immunological and genetic mechanisms, offering a transformative framework for predictive biomarkers and personalised therapies that could redefine IPF management across diverse populations.

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P09.030.D Catalogue of HBB variants: the significance of harmonising HBB nomenclature in variant classification

Dino Hasanagic[1], *Melida Mahinic*[1], *Ana Gutalj*[1], *Kathryn Curry*[2], *Ena Toman*[1], *Maida Ljubijankic*[1], *Betty Mattias*[2], *Jane Beckwell*[2], *Jeffrey Bissonnette*[2], *Denis Trivanovic*[1], ***Amina Kozaric***[1], *Mark J. Kiel*[2]

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Background: The HBB gene encodes the beta-globin subunit of haemoglobin and variants in this gene are associated with various haemoglobinopathies, including sickle cell disease and beta-thalassemia. The inconsistency in *HBB* variant nomenclature has contributed to challenges in accurate interpretation and classification.

Material and Methods: Mastermind Genomic Search Engine (Genomenon, Inc) was used to collect all *HBB* variants from the literature, followed by a semi-automated curation with manual expert review to classify variants according to the ACMG/AMP criteria. Each variant was initially linked to its published classification; however, reclassification was performed following adjustments to the nomenclature.

Results: Systematic literature review of 3,234 articles yielded 1,480 *HBB* variants. Out of those, 141 (9.5%) HBB variants required nomenclature revisions. After reclassification, 28 variants were upgraded to pathogenic (eight variants were previously classified as conflicting interpretations of pathogenicity in ClinVar and 20 were VUS), 6 variants were downgraded to likely benign from conflicting, while 69 variants remained VUS. Examples of upgraded variants are: p.Ala130Pro (Hb Crete), p.Glu102Gln (Hb Rush), and p.Glu122Lys (Hb O-Arab, also known as Hb Egypt and Hb O-Thrace).

Conclusion: The application of proper *HBB* variant nomenclature led to clinically significant changes for 34 variants. This effort underscores the importance of establishing standardised nomenclature and classification practices to improve the accuracy and consistency of genetic variant interpretation, which is crucial for clinical decision-making and patient care.

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P09.031.A De Novo or Inherited: Gonosomal Mosaicism in Hereditary Angioedema due to C1 Inhibitor Deficiency

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Background: Hereditary angioedema (HAE) is a rare genetic disease characterized by recurrent episodes of swelling due to dysregulated endothelial permeability. The most common form, HAE-C1-INH, results from pathogenic genetic variants in the SERPING1 gene, leading to deficient C1-Inhibitor function and transmitted in an autosomal dominant manner.

Materials and Methods: We investigated a patient with sporadic HAE-C1-INH presenting with typical clinical features and markedly reduced C1-INH function. Sanger sequencing was performed in the index patient and the asymptomatic parents with normal C1-INH function and C4 levels. cDNA sequencing evaluated the effect of the identified variant on splicing. Deep amplicon sequencing of maternal DNA was used to quantify mosaicism across tissues.

Results: We identified a novel splice site variant in SERPING1 (c.890-1G>C) and, by analyzing the cDNA, we demonstrated that the variant produces two aberrantly spliced transcripts, confirming its pathogenicity. Despite the normal function of C1-INH in the parents, we found that unexpectedly, the mother was a carrier of the genetic variant. Sanger sequencing profiles revealed the presence of gonosomal mosaicism in the mother. We confirmed and quantified the mosaicism in different tissues by high-depth NGS-based deep amplicon sequencing, showing a variant allele frequency ranging from 17 to 23%.

Conclusion: In this study, we present the first case of gonosomal mosaicism in a family with a single child affected by HAE-C1-INH and unaffected parents. Our results underscore the importance of parental genetic testing in all patients, even if asymptomatic, and highlight the implications of gonosomal mosaicism for genetic counseling.

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P09.032.D Optimisation and validation of a droplet digital PCR assay for copy number variation of the alpha-tryptase coding region of *TPSAB1* to identify hereditary alpha-tryptasemia

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Background: Testing for hereditary alpha-tryptasemia (HαT) is now available to patients in England with persistently elevated basal serum tryptase. Identifying HαT informs patient management, limits unnecessary testing for more serious blood disorders and prevents excessive treatment, NHS cost and ongoing patient anxiety[1]. HαT is associated with an in *cis* copy number increase of the alpha-tryptase coding region of *TPSAB1* (*TPSAB1α*)[2].

Material and Methods: Clinical validity of a droplet digital polymerase chain reaction (ddPCR) protocol, provided by Manchester University NHS Foundation Trust, for *TPSAB1α* copy number to identify HαT was assessed. This included using digested DNA and undigested DNA, to facilitate alpha shift calculations (copy number in digested DNA - copy number in undigested DNA) and distinguish *TPSAB1α* copies in *cis* from copies in *trans* when *TPSAB1α* absolute copy number is two. Fifty-two samples of known *TPSAB1α* copy number were blind tested using an optimised protocol.

Results: The protocol was highly accurate for determining *TPSAB1α* copy numbers up to four (including zero) but less accurate at higher copy numbers. Ranges for defining *TPSAB1α* copy number based on the observed copy number values were set accordingly. Alpha shift thresholds were also set.

Conclusion: Following local adjustments and refinement, a protocol for *TPSAB1α* copy number detection by ddPCR has been validated for clinical use. Ongoing data monitoring will be carried out to show protocol efficacy on a larger data set. We discuss how this technique could have other clinical applications where phasing of duplicated regions informs clinical outcome or risk.

C.O.I. Disclosure:

H. Gartland: None.

P09.033.C Genetic profiling of myeloid malignancies: A single-center study using myeloid panel testing

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Background: Myeloid neoplasms are a genetically heterogeneous group and the detection of associated genetic alterations plays an important role in the differential diagnosis, prognosis and treatment of the disease. For this reason, myeloid panel studies using next-generation sequencing (NGS) are of great importance in this patient population. In this study, we aim to contribute to the literature by summarising the results of myeloid panel studies in our center.

Material and Methods: Between June 2024 and January 2025, 98 patients referred to our clinic were tested using a large customised myeloid panel containing 65 genes. Patients with acute myeloid leukaemia (AML) represented the largest group with a total of 46 (46.9%) patients. 26 (26.5%) patients with chronic myeloproliferative disorders (CMPD) and 16 (16.3%) patients with myelodysplastic syndrome (MDS) also made up the majority of the study group with AML patients (89.7% in total).

Results: A total of 161 oncogenic/likely oncogenic variants were detected in 82/98 (%83.6) patients according to the ClinGen/CGC/VICC criteria. The most common variants in AML patients were in *DNMT3A, NPM1, NRAS*, and *FLT3*; in CMPD patients in *NF1*, *ASXL1*, and *NRAS*; and in MDS patients in *RUNX1*, *ASXL1*, and *CBL*.

Conclusion: Due to the genetic heterogeneity of myeloid neoplasms, it is important to use large panels in this group and to clarify and manage conditions such as germline variants, mosaicism or clonal haematopoiesis of indeterminate potential (CHIP) by evaluating some features such as the fractions of detected variants, age and clinical findings of the patients.

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P09.034.D Investigating diagnostic efficacy of whole exome sequencing in inborn errors of immunity

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Background: Inborn errors of immunity (IEI) represent a highly heterogeneous group of disorders, encompassing over 400 potential causative genes, making genetic diagnosis a challenging process. The identification of genetic background underlying IEIs has significantly advanced with the widespread adoption of next-generation sequencing (NGS) technologies. However, diagnostic yield remains around 30%. This study aims to collect and analyze whole exome sequencing (WES) data from patients with a phenotype of IEI to assess diagnostic efficacy in our laboratory.

Material and Methods: WES was conducted using established standard protocols. Sequencing data were analyzed through our bioinformatics pipeline with a newly developed virtual gene panel, which included all genes associated with IEI as defined by the International Union of Immunological Societies (IUIS). Variant classification was performed according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results: Of the 228 samples tested, clinically relevant genetic variants were identified in 46 cases, resulting in a diagnostic efficacy of 20%. Several novel genetic variants, classified as likely pathogenic, were detected. The 46 diagnosed patients were categorized into the disease groups of the IUIS classification, with representation from each major group, reflecting the broad spectrum of IEIs in the cohort.

Conclusion: Our study demonstrates that WES provides a valuable tool for the genetic diagnosis of IEIs, achieving a diagnostic efficacy of 20%. This yield correlates well with data from similar studies; however, it remains lower than that observed for other genetic diseases, highlighting the need for improvement and potential implemantation of complementary diagnostic methods.

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P09.035.A The dilemma of X-linked agammaglobulinemia (XLA) carriers:multicenter, retrospective cohort study on the reproductive choices of XLA families

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Background: Many X-linked agammaglobulinemia (XLA) patients have reached adulthood, with female relatives in reproductive age possibly carrying a deleterious *BTK* variant. Studies on reproductive outcomes in XLA families are lacking. We sought to investigate the state of preconception/prenatal genetic counseling, carrier status screening, and reproductive decisions in XLA families.

Material and Methods: In this multicenter, retrospective cohort study, we collected a 3-generation pedigree and data on mothers/sisters of XLA index patiens, including carrier status and pregnancy outcomes.

Results: 53 adults with XLA, belonging to 52 families were gathered. 52 mothers, and 33 sisters were enrolled. All XLA sisters received genetic counseling, 40% opting for carrier status determination. The main reasons for declining genetic testing in XLA sisters were their young age and the perception of XLA as an affordable disease, followed by the willingness to postpone the decision at the time of pregnancy, and by the decision to not have children. Among the carriers in reproductive age 60% chose invasive prenatal diagnosis. Prenatal testing resulted in 5 XLA diagnoses, with 2 pregnancy terminations, 1 miscarriage, 2 live births. 3 carriers refused prenatal testing, with 6 live births, including 3 XLA-affected sons. One non-genotyped sister had an XLA affected son. In total, 9 XLA diagnoses were made, including 6 live births.

Conclusion: Attitutes towards preconception and prenatal testing in XLA families are varied. Preconception genetic counseling, and the perception of XLA as a manageable conditions with dedicated care are percieved as key factors for informed choices by XLA families.

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P09.037.C Role of founder effect in forming a new endemic region of patients with thiamine-responsive megaloblastic anemia

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Background: Rogers syndrome, or thiamine-responsive megaloblastic anemia (TRMA), is a hereditary disorder caused by dysfunction of the high-affinity thiamine transporter 1. This syndrome is characterized by a clinical triad of symptoms: megaloblastic anemia, non-autoimmune diabetes mellitus, and sensorineural hearing loss. To date, approximately 100 families with genetically proven disease have been documented, primarily in endemic regions such as the Middle East, South Asia, and the northern Mediterranean. Notably, there have been no reported cases in Russia. This research aims to clinically characterize patients with genetically confirmed Rogers syndrome and investigate the founder effect's role in its increased incidence in Russia.

Material and Methods: A total of ten patients diagnosed with TRMA were analyzed, with a median age at diagnosis of 2 years (ranging from 1 to 16 years).

Results: Symptoms manifested from infancy to the first year of life; seven patients exhibited the classical triad, while others presented additional symptoms like cardiomyopathy. Genetic testing revealed a pathogenic homozygous c.1223+1G>A mutation in the *SLC19A2* gene in eight of the patients, all of whom reside in the North Caucasian federal district. This finding suggests a potential founder effect contributing to the higher frequency of TRMA in this region. Furthermore, it indicates that the North Caucasus may be a new endemic region for this disease.

Conclusion: Timely diagnosis and treatment are essential to prevent severe complications associated with this disorder. Identifying new endemic areas TRMA may facilitate earlier recognition and management of affected children.

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P09.038.D GenIA, the Genetic Immunology Advisor database for genetically driven immune disorders

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Background: The landscape of genetically driven immune disorders has grown rapidly over the last decades thanks to the combination of hypothesis-generating -omics technologies and hypothesis-driven functional studies. However, with increasing knowledge comes the challenge of capturing, harmonizing and synthesizing all layers of genotypic, phenotypic and mechanistic information while making this easily accessible.

Objective: To create a comprehensive, expertly curated, multidimensional database that enables aggregation, standardization, and synthesis of knowledge in the scientific literature by and for researchers, geneticists and clinicians in the field.

Methods: The database was designed and written in SQL following a subject-centered model. The web application is written in PHP, HTML, CSS and JavaScript. Most of the data stored in GenIA was extracted by manual review of research articles, though unpublished functional data can also be stored.

Results: We developed a publicly accessible resource that provides curated, detailed and multilayered information, anchored around patients; but also includes diagnostic paradigms and pitfalls associated with individual genes and conditions, along with an international functional test registry. Literature curation was completed for 30 genes and is ongoing for others. These pilot curations show the many ways in which this database can be used by scientists and clinicians to answer specific and valuable questions.

Conclusion: GenIA encompasses more thorough and comprehensive genetic, mechanistic, and phenotypic immunogenetic knowledge than other currently existing databases. GenIA's architecture allows sophisticated data interrogation, the result of which may ultimately translate into reduced diagnostic odysseys, better preventing strategies and improved patient care. Our model could be adopted by other clinical areas.

C.O.I. Disclosure:

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P09.039.A HLA-DRB1 high-resolution allele frequencies in a cohort of Romanian stem cell donors

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Background: The HLA-DRB1 alleles are one of the primary molecular representatives of the HLA-class gene cluster. The aim of the current study is identifying the main HLA-DRB1 alleles in a Romanian cohort of healthy stem cell donors and their 6-digits high-resolution frequencies.

Material and Methods: The study, conducted at the Immunology and Transplantation Immunology Department of the Fundeni Clinical Institute, involved 420 voluntary donors from the Romanian National Registry of Voluntary Hematopoietic Stem Cell Donors (RNDVCSH). HLA genotyping was carried out using the Immucor Mia Fora NGS MFlex kit.

Results: The results revealed 41 different HLA-DRB1 alleles, the most common being DRB1*16:01:01 (allele frequency, AF, 12.6%), DRB1*11:04:01 (AF 12.1%), DRB1*03:01:01 (AF 12%), DRB1*07:01:01 (AF 9.3%), DRB1*01:01:01 (AF 7.3%) and DRB1*11:01:01 (AF 6.3%) and 13:01:01 (AF 6%). Out of all the detected HLA-DRB1 variants, only 3 showed frequencies higher than 10%, while 18 had frequencies lower than 1%, out of which 15 were rare variants, with AFs lower than 0.5% (identified in maximum 3 of the 420 individuals each).

Conclusion: The current research provides important data on the HLA-DRB1 alleles frequencies for the Romanian population. The study showed similarities as well as disparities for the more common and less frequent alleles between our analyzed cohort and the various European and non-European populations, proving the importance of this data for the specificity of organ transplantation among different populations and individuals, as well as for a better understanding of the population genetics of the region. Further studies are needed for confirming these findings.

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A. Caragea: None. **R. Ursu:** None. **A. Constantinescu:** None. **L. Bohiltea:** None. **I. Constantinescu:** None.

P09.040.B Differential gene expression analysis of iNKTs cultured for 4 and 6 weeks

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Background: Invariant NKT cells (iNKTs) are rare regulatory cells that are increasingly used in cell therapy (e.g., to treat severe infections or prevent post-transplant complications). They can be used allogeneically, which allows us to make a batch of cells from one apheresis that can be used for multiple patients. To treat as many patients as possible, we need to expand them in vitro. However, long-term culture can lead to changes in the transcriptome that are associated with a reduction in the therapeutic potential of the cells. Our aim was to compare the transcriptome of iNKTs cultured for the minimum and maximum time periods used in clinical settings.

Material and Methods: iNKTs from 7 healthy donors were isolated using microbeads and activated with IL-15, CD1d positive feeder cells (C1R-CD1d) and α-galactosylceramide for 4 and 6 weeks. Total RNA was extracted and rRNA depleted. The final libraries were normalised, pooled and sequenced.

Results: PIAS4 and IL17D gene expression was significantly downregulated, whereas the key signaling receptors CD28, CD3G, IL2RA and IL6ST were significantly upregulated after six weeks of cultivation. Upregulation of IL41I, associated with lower T cell proliferation activity was also detected. GOE analysis revealed biological pathways such as T cell activation, immune response regulation and activation pathway and T cell receptor signaling pathway. Gene expression of key senescence genes remained statistically unchanged over the culture period.

Conclusion: Prolonged cultivation of iNKTs has no adverse effect on the transcriptional activity, allowing us to expand the cells in larger numbers.

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P09.041.C Fetal Down Syndrome: An Early Analysis of AIRE and GATA1-Related Immunologic and Hematologic Dysfunctions

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Background: Down syndrome (DS), caused by trisomy of chromosome 21, is associated with immune and hematological dysfunctions, including thymic abnormalities. The latter contribute to the dysregulation of the immune system, increasing susceptibility to autoimmune diseases and infections. The *AIRE* (Autoimmune Regulator) gene expression can compromise immune tolerance, leading to abnormal autoimmune responses in DS. Similarly, the *GATA1* gene is mutated in DS, favouring hematological disorders such as anemia and megakaryocytic leukemia. *GATA1* is also expressed in thymic cells, influencing T cell development and function. *GATA1* expression modulation plays a critical role in the development of leukemia in DS. To the best of our knowledge, in DS fetuses, abnormalities in *GATA-1* and *AIRE* expression have never been analyzed in depth.

Material and Methods: Formalin fixed paraffin embedded (FFPE) tissues (thymus, liver, heart) from 3 DS fetuses of the second trimester obtained from legal termination of pregnancy and 3 age matched controls were used for gene expression analysis. RT-qPCR was performed to analyze *AIRE* and *GATA1* expression at a precocious stage. *GATA1* mutations analysis was performed on Sanger sequencing.

Results: RT-qPCR analysis identified the differential expression of *AIRE* e *GATA1* genes between DS fetuses and controls. These results substain the hypothesis of precocious immune and hematological dysfunctions that impair the fetuses health.

Conclusion: The findings of these early dysfunctions in DS immune system may pave the way for new diagnostic and

therapeutic strategies, allowing early intervention in pregnancy and better management of DS-associated issues in life.

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P09.042.D Investigation of Genetic Regulation of Paroxysmal Nocturnal Hemoglobinuria Disease

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a ultra rare clonal stem cell disease that manifests itself with acquired non-immune intravascular hemolysis findings, bone marrow failure, and thrombosis clinic. Since PNH occurs as a result of somatic mutation of hematopoietic stem cells, it affects all three cell lines of the hematopoietic system at different rates.

This study aims to elucidate the molecular mechanisms underlying the rare disease PNH, to obtain more data on this patient group, and to investigate expression levels of some epigenetic regulator genes and disease-associated gene.

Material and Methods: Blood samples were collected from 21 PNH patients. RNA isolation and cDNA conversion were performed for further analyses. mRNA expression levels of DNMT3A, ASXL1, TET2, EZH2, RUNX1, IDH1, PIGA, PHF6, U2AF1, ATM genes were determined using qRT-PCR method. Quantitative evaluation of gene expressions was performed by ΔΔCT analysis using the housekeeping GAPDH gene ratio. $p < 0.05$ was considered significant.

Results: Overexpression was observed in DNMT3A, ASXL1, TET2, EZH2, RUNX1, PHF6, ATM and U2AF1 genes. Fold change values were detected as 1.04, 15.30, 1.57, 1.87, 3.58, 2.38, 3.43 and 3.94, respectively. Downregulation was observed in IDH1 and PIGA genes. Fold change values were detected as 0.86 and 0.15, respectively.

Conclusion: Overexpression was observed in most of the methylation-related genes and especially the overexpression in ASXL1 gene suggests that this gene may play an important role in the diagnosis and treatment of the disease. Further studies, such as WES and RNA-seq, will be performed for better understanding of pathogenesis of the disease.

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P09.043.A Spontaneous hematological recovery in a patient with Fanconi Anemia

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Background: Fanconi anemia (FA) is a rare autosomal recessive disorder caused by pathogenic variants in DNA-repair genes, leading to bone marrow failure, congenital abnormalities and cancer predisposition. Somatic genetic rescue via revertant mosaicism has been reported, particularly in hematopoietic tissues.

Material and Methods: We describe two siblings with FA who exhibit discordant phenotype severity and genotype.

Results: A 16-year-old boy presented short stature, ectopic kidney, thumb duplication, a positive DEB-test and abnormal cell cycle analysis. He underwent hematopoietic stem cell transplantation (HSCT) at the age of 5 years due to progressive pancytopenia. His 13-year-old sister had multiple café-au-lait spots, thumb duplication, a positive cell cycle test at 1.5 years, and thrombocytopenia at 2 years, for which HSCT evaluation was initiated. However, her thrombocytopenia resolved spontaneously, and she remains hematologically stable. Clinical exome analysis revealed that the boy carried the heterozygous *FANCA* c.3660del, p.(Asn1221fs*26) and c.50dup, p.(Arg18Profs*19) variants, while his sister carried the heterozygous c.3660delC; p.(Asn1221fs*26) and c.51C>G p.(Gly17=) variants. Notably, the synonymous c.51C>G variant occurred at the same codon where the brother had the frameshift variant c.50dup, suggesting a possible somatic genetic rescue in hematopoietic tissue potentially explaining her hematological recovery.

Conclusion: Revertant mosaicism has diagnostic and clinical implications, necessitating repeated genetic analyses across tissues and over time. Spontaneous hematological remission should not reduce follow-up for other FA manifestations, as it remains a systemic disease, with persistent risks of other complications.

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P09.044.B Prevalence, molecular and laboratory features of beta-thalassemia carriers in Northern part of Russia

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Background: Most cases of beta-thalassemia are diagnosed in regions with high prevalence of malaria. Russia is not endemic for malaria, but since it is a multi-ethnic country, it is believed that the prevalence of beta-thalassemia may be high.

Material and Methods: For characterization of prevalence, molecular and laboratory features of beta-thalassemia, results of blood test (RBC, Hb, hematocrit, MCV, MCH, MVHC, RDW) of 58266 risk group patients from Northern part of Russia were analyzed. For cases with low Hb and/or MCV and/or MCH, Mentzer and Sirdah indices were calculated. For samples positive for both indices, sequencing of *HBB* gene was performed.

Results: In studied group 182 samples were positive for both indices. After genotyping 64 samples were positive for 20 different heterozygous pathogenic *HBB* variants. The most common variant was c.25_26del (25.81%), but prevalence of others did not exceed 10% (c.93–21G>A - 9,68%, c.92+1G>A - 8.06%, c.316-197–8.06%, c.27dup - 8.06% etc.). Distribution of variants was uniform in coding and non-coding regions of *HBB*. Prevalence of *HBB*-positivity was 0.11%. *HBB*-positive patients had lower level of RBC,

Hb, MCV, hematocrit, MCH, RDW, indices and higher level of MCHC than *HBB*-negative patients ($p < 0.0001$). Prevalence of anemia in *HBB*-positive samples was 64%, in *HBB*-negative - 37%.

Conclusion: A high prevalence of beta-thalassemia carriers has been identified. There was only one hotspot variant (c.25_26del) with low frequency (25.81%). Laboratory features highlight that not all cases of beta-thalassemia present with anemia, and HBB-positive cases are characterized by more profound changes in blood test results and indices.

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P09.045.C Investigating the shared genetic architecture between Immunoglobulin A deficiency and autoimmune diseases

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Background: Immunoglobulin A deficiency (IgAD) is the most prevalent primary immunodeficiency, characterized by low serum IgA levels. Autoimmune diseases (ADs) frequently co-exist with IgAD, suggesting a shared genetic basis. This study aims to explore the genetic architecture underlying IgAD and ADs, shedding light on potential shared mechanisms.

Material and Methods: We utilized summary statistics from large-scale genome-wide association studies (GWAS) of IgAD and multiple ADs. Employing multi-trait analysis, genetic correlation, and co-localization analysis, we identified pleiotropic loci shared between IgAD and ADs. Shared genes between IgAD and ADs were identified through TWAS and MAGMA analysis, followed by functional enrichment analysis to uncover shared biological pathways and potential drug targets. Heritability enrichment and single-cell RNA-seq analysis were used to identify the critical tissues and cell types involved in both conditions.

Results: Our analysis identified 54 pleiotropic loci and 89 pleiotropic sigals significantly associated with both IgAD and ADs ($p < 5 \times 10^{-8}$). Co-localization analysis highlighted shared loci, including IFIH1, AHI1, CLEC16A, and STAT4. Gene-level analysis revealed shared pathways related to T-cell differentiation, viral infection, immunological diseases, and the intestinal immune network for IgA production. Heritability enrichment analysis pinpointed whole blood and the intestine as critical tissues for both conditions and single-cell RNA-seq analysis identified B cells as the most affected cell types.

Conclusion: Our findings unveiled a shared genetic architecture between IgAD and ADs, highlighting common functional mechanisms and providing insights into the biological interplay between these conditions.

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P09.047.A IGLL1 in Newborn Screening for Severe T and B Cell Lymphopenia Using TREC/KREC Detection

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Background: Agammaglobulinemia due to variants in *IGLL1* thought to be extremely rare form of B-cell deficiency but recently newborn screening by T cell receptor excision circle (TREC) or kappa-deleting recombination excision circles (KREC) revealed it to be much more frequent.

Material and Methods: TREC/KREC detection was performed more than 2 millions newborns from all regions of Russian Federation in 24-month period. If TREC or KREC were low than flow cytometry for lymphocyte subsets was done. 86 infants pretended to have agammaglobulinemia due to low counts of B-cells and KREC levels. In these 86 infants WES was done to find genetic defect. All of the genetic findings were confirmed by Sanger sequencing.

Results: In 22 newborns were found pathogenic or likely pathogenic genetic variants in *IGLL1* in homozygous or compound heterozygous form. Mostly, in 16 cases c.425C>T p.(Pro142Leu) [NM_020070.4] was found in homozygous (in 9 cases) or compound heterozygous (with other mutations or deletion of 1–2 exon) form. We observed high prevalence of this mutation in infants with low counts of B-cells and low KREC levels.

Conclusion: Formely, IGLL1 is the most frequent cause of isolated low KREC in newborn screening, but it's not as severe as we expected. Patients and sometimes their homozygous parents or sibs have no clinic of agammaglobulinemia.

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P09.049.C The importance of whole genome sequencing in rare immunodeficiencies - case report of a pair of twins with Cartilage hair hypoplasia with Omenn syndrome

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Background: Primary immunodeficiency diseases (PIDs) are clinically and genetically highly heterogeneous with ~500 causative genes. Genetic diagnosis is challenging, whole exome sequencing (WES) might not lead to correct molecular diagnosis even in monogenic forms.

Patients and Methods: The proband and her twin sister were suspected to have Omenn syndrome, based on immunological parameters and clinical symptoms (lymphadenopathy, eosinophilia, dysmorphic appearance, low nasal bridge, shortening of tubular bones, generalized erythrodermia). WES analysis and array CGH did not reveal any pathogenic variant. In order to establish the diagnosis, whole genome sequencing (WGS) was performed in the deceased twin pair and parents.

Results: WGS identified compound heterozygosity for two previously reported pathogenic variants (LRG_163(t1):n.1-22_1-3dup and LRG_163(t1):n.147G>A) in the non-protein coding *RMRP* gene. Based on the genetic findings and the clinical picture, the diagnosis of Cartilage-hair hypoplasia with Omenn syndrome was established in the siblings.

Conclusion: The diagnostic efficiency of WES in case of primary immunodeficiency is very low (~20%). In the case of negative WES result, whole genome sequencing can be a significant aid in making a genetic diagnosis as it covers mitochondrial genome,

non-coding regions, promoters, enhancers, introns, regulatory elements and can detect CNVs and other structural variants. If the genetic background is identified, prenatal or pre-implantation testing should be offered.

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P09.050.D A novel homozygous deletion of exon 10 of *PKLR* gene as a cause of severe congenital transfusion dependent hemolytic anemia

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Heart and Brain Center of Clinical Excellence, Pleven, Bulgaria.

Background: Pyruvate kinase deficiency (PKD) is the most common chronic hereditary non-spherocytic hemolytic anemia. The disease is characterized by variable degree of severity of chronic hemolytic anemia, from mild asymptomatic anemia to life-threatening, transfusion-dependent anemia. Chronic complications resulting from hemolysis, include iron overload, decreased bone density, cardiopulmonary complications, and increased overall mortality. We present a case of 1-year-old boy affected by severe congenital hemolytic anemia that needs many successive haemotransfusions since its early neonatal age due to laboratory confirmed severe and life-threatening hemolysis. The proband is the fourth child of consanguineous parents whose firstborn baby girl died with symptoms of severe hemolytic anemia.

Material and Methods: The genetic background of the condition of the proband, confirming PKD in the family, was elucidated by NGS-based method combined with computational CNV analysis, subsequently confirmed by orthodoxal method.

Results: Whole exome sequencing (WES) analysis revealed a novel pathogenic homozygous deletion covering exon 10 of the *PKLR gene* (NM_000298.6) encoding the pyruvate kinase enzyme. While there are several well described single nucleotide variants in the region that cause loss of function and are related to disease, reported phenotypes contributed to CNVs affecting exon 10 of the gene are missing.

Conclusion: We provide a detailed overview of a proband with a rare genetic form of PKD, describing the phenotype, family history and challenges of diagnostics in order to the need for thorough molecular analysis in patients with undiagnosed hemolytic anemia. We discuss the available treatment options for the conditions and the outcomes for our patient.

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P09.051.A Novel *TLR7* frame-shift mutation probably associated with multiple sclerosis: functional characterization and transcriptome analysis

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Background: Toll-like receptor 7 (*TLR7*) plays a crucial role in the innate immune system by recognizing viral RNA and activating pathways that induce IRF7 and NF-κB. Variants in *TLR7* have been linked to autoimmune diseases like SLE and severe COVID-19 outcomes. In this study, we report a novel frame-shift mutation in *TLR7* in a female patient with multiple sclerosis (MS). We aimed to investigate the functional consequences of this mutation through transcriptome analysis and in-vitro PBMC stimulation experiments.

Material and Methods: Whole-genome sequencing revealed a heterozygous *TLR7* frame-shift variant (c.2441del, p.Cys814Leufs*12) in the patient and her father who was found to be hemizygous for the mutation after targeted sequencing. Whole transcriptome sequencing was performed on blood samples from the patient, her father, and 4 TLR7-wild-type controls. Differential expression analysis was conducted with DESeq2 (Q value ≤ 0.05). PBMCs of patient and controls were stimulated in vitro with Imiquimod and R848 to assess IRF7 and NF-κB gene expression using RT-qPCR, and the results were analyzed using GeneGlobe software (QIAGEN).

Results: Transcriptome analysis identified 65 differentially expressed genes: 34 up-regulated and 31 down-regulated. Enrichment analysis suggested a dysregulated immune response, with up-regulated genes such as *CD14*, *IL12RB2*, and *IL18RAP* involved in immune activation. Down-regulated genes like *TLR7*, *PAX5*, and *CD79A/B* are crucial for B-cell signalling.

Conclusion: This *TLR7* frame-shift mutation may contribute to immune dysregulation in mutation carriers. The altered expression of immune-related genes points to potential mechanisms linking *TLR7* variants and autoimmune diseases, warranting further study.

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P10 Intellectual Disability

P10.001.B Biallelic variants in *RELCH*, encoding a Rab11-binding protein, cause a complex neurodevelopmental disorder

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Background: RELCH is a Rab11-GTP binding protein involved in nonvesicular cholesterol transport from recycling endosomes to the trans-Golgi network. To date, *RELCH* has not been implicated in human disease.

Material and Methods: We used a multidisciplinary approach that combined clinical information and exome sequencing data with in vitro and in vivo modeling to identify the underlying genetic cause and disease pathomechanism.

Results: We investigated five pediatric cases from two unrelated consanguineous families that were unified through GeneMatcher. Our cases presented with severe neurodevelopmental phenotypes, including global developmental delay, microcephaly, generalized spasticity, and profound neurological impairments. MRI scan revealed ventriculomegaly, abnormalities in the corpus callosum, and bilateral gyral dysmorphism in occipital lobes with encephalomalacia in left occipital lobe. Exome sequencing identified a biallelic frameshift variant in family 1 while family 2 harbors a splice donor site variant in *RELCH*; both variants segregate with the phenotype in each respective family. Patient-derived primary fibroblasts revealed no detectable protein in family 1, and exclusion of exon 4 with concomitant reduced immunoreactivity of RELCH in family 2. Furthermore, zebrafish models with *relch* ablation display neurodevelopmental deficits including reduced head size and a depletion of commissural neurons.

Conclusion: Genetic and functional data implicate *RELCH* loss-of-function with a clinically heterogeneous neurodevelopmental disorder characterized by microcephaly and profound neurological impairments. Thus, we add to the repertoire of genetic disorders underscored by deregulated intracellular cholesterol.

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P10.002.C Clinical and functional studies of a complex neurodevelopmental disorder caused by variants in CTNND2

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Background: Heterozygous variants in *CTNND2* are associated with a broad spectrum of neurodevelopmental disorders, ranging from mild (dyslexia, ADHD) to severe (intellectual disability, autism). Despite its clinical significance, a comprehensive characterization of *CTNND2*-associated disorders is lacking. Furthermore, δ-catenin, encoded by *CTNND2*, is a brain-specific component of the cadherin-catenin cell adhesion complex, but it remains unclear how its loss affects neurodevelopment.

Material and Methods: We analyzed the clinical and genetic profiles of 54 patients carrying 40 *CTNND2* variants, identified via exome sequencing, SNP array, and karyotyping. Facial dysmorphism was evaluated using Face2Gene, and pathogenicity was assessed through in-silico modeling. Functional studies included in vitro neural modelling and transcriptome analysis of three patient-derived neural stem cell lines and organoids to investigate early neurogenesis. CRISPR-Cas9 was employed to generate three *CTNND2* knockout lines for comparative analysis.

Results: The *CTNND2* variants, missense ($n = 14$) and loss-of-function ($n = 26$), were linked to developmental delay (61%), intellectual disability (65%), and behavioral abnormalities (52%). In cases with family data, 11 had de novo mutations, and 4/16 transmitting parents showed a phenotype. Functional studies, though variable between lines, revealed disrupted neurogenesis in one patient, characterized by aberrant neural rosette formation. Transcriptome analysis showed dysregulated WNT signaling. Partial rescue of these phenotypes was achieved by excluding a WNT activator, highlighting δ-catenin's role in early neural development.

Conclusion: Our study highlights the phenotypic and genetic heterogeneity of *CTNND2*-related disorders, emphasizing δ-catenin's critical role in neurodevelopment. WNT signaling dysregulation suggests a potential mechanistic pathway, underscoring the need for personalized diagnostic and therapeutic approaches.

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P10.003.D Non-coding structural variants disrupt regulatory elements steering FOXG1 transcription in early neurodevelopment (Award Candidate)

Lisa Hamerlinck[1], *Eva D'haene*[1], *Nore Van Loon*[1], *Michael B. Vaughan*[1], *María del Rocío Pérez Baca*[1], *Lara Colombo*[1], *Sebastian Leimbacher*[1], *Lies Vantomme*[1], *Esperanza Daal*[1], *Annelies Dheedene*[2], *Björn Menten*[2,1], *Bert Callewaert*[2,1], *Sarah Vergult*[1]

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Background: The FOXG1 transcription factor is a crucial regulator of embryonic brain development. Pathogenic variants affecting FOXG1 cause FOXG1-syndrome. Although structural variants (SVs) downstream of FOXG1 have been reported in individuals with FOXG1-syndrome-like characteristics, the regulatory mechanisms leading to aberrant FOXG1 transcription remain unclear.

Methods & Results: We performed a thorough literature review and identified 37 individuals with FOXG1-syndrome-like characteristics and an SV affecting the noncoding sequence downstream of FOXG1. Furthermore, we identified a de novo noncoding deletion in an individual with FOXG1-syndrome features, allowing us to define a ~100 kb commonly affected regulatory region (CARR). By integrating publicly available and in-house epigenomics data, Hi-C, UMI-4C, and in vivo enhancer assays in zebrafish, we uncovered multiple regulatory elements within this CARR, including a neuronal enhancer cluster and the distal boundary of the FOXG1-containing topologically associating domain (TAD). Hi-C analysis on a lymphoblastoid cell line of our patient revealed increased interactions with the adjacent TAD. Moreover, sequential UMI-4C and CUT&RUN assays during induced pluripotent stem cell (iPSC) differentiation into neural progenitor cells (NPCs) demonstrated dynamic activation of the

enhancer cluster and increased interaction with the FOXG1 promoter. Finally, CRISPR-Cas9 deletion of the enhancer cluster and TAD boundary in iPSC-derived NPCs resulted in decreased FOXG1 transcription.

Conclusion: We identified and characterized a CARR downstream of FOXG1, containing enhancer and architectural elements essential for proper FOXG1 transcription. Our findings provide new insights into chromatin architecture and gene regulation at the FOXG1 locus, improving SV interpretation in individuals with FOXG1-syndrome-like characteristics.

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P10.004.A Competition in blood of clones with both loss-of-function and hypomorphic or benign *NIPBL* variants in Cornelia de Lange patients: postzygotic mutations or rescue events? (Award Candidate)

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Background: Cornelia de Lange (CdLS) is a rare recognizable intellectual disability syndrome. Loss-of-function (LOF) *NIPBL* variants explain ~70% of solved cases. Most pathogenic variants occur de novo and 13% are mosaics with a somatic/blood allelic ratio (AR) bias that remains poorly understood and impedes blood-based tests.

Material and Methods: We report rates of *NIPBL* mosaic variants in a 10-year retrospective analysis of CdLS patients screening. In two cases with two mutant populations with a WT and two alternative exclusive mosaic alleles, we performed single-molecule deep (1000x-average) sequencing in blood ± saliva ± skin fibroblasts, RNAseq, and/or long-read HiFi PacBio sequencing.

Results: Of 97 pathogenic *NIPBL* variants carriers, 24 (24.7%) showed mosaicism. No nonsense/frameshift variant was detectable in blood (AR < 1%) while 3 missense, 1 structural variant (SV), 3 splice (including 1 deep-intronic) variants were detectable in blood (AR = [4–25%]), suggesting a hypomorphic effect.

In two cases, two mutant populations were identified. One showed one haplotype carrying either a deep intronic one-bp deletion resulting in a 53-bp frameshift inclusion in RNAseq from cultured fibroblasts or a nearby variant with no effect on splicing, enriched in blood. The second patient carried a 2-exon in-frame deletion in blood with corresponding exon skipping, and multiple low-level breakpoints. PacBio sequencing revealed that there were two clones from one haplotype, the second clone harbored a low-level complex frameshift SV, which was likely the causal event, both having likely occurred during the zygote division.

Conclusion: Postzygotic mutations and rescue of mutant alleles may explain somatic/blood biased AR, along with NIPBL functions in mitosis.

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P10.005.B Further characterization of *BRSK2*-associated neurodevelopmental disorder (Award Candidate)

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Florida, Tampa, FL, USA, [33]Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Center, Fildelfia, Dianalund, Denmark, [34]Institute of Humangenetics, Faculty of Medicine, University Hospital Cologne, Cologne, Germany, [35]Institute of Medical Genetics, University of Zurich, Schlieren, Zurich, Switzerland, [36]Research Priority Program (URPP) ITINERARE: Innovative Therapies in Rare Diseases, University of Zurich, Zurich, Switzerland, [37]Neuroscience Center Zurich, University of Zurich and ETH Zurich, Zurich, Switzerland, [38]Servicio de Neurología Infantil, Sección de Neurogenética, Hospital Universitario Quirónsalud, Madrid, Spain, [39]Facultad de Medicina, Universidad Europea de Madrid, Madrid, Spain, [40]Arcensus GmbH, Rostock, Germany, [41]CHU Bordeaux, Service de Génétique Médicale, F-33000, Bordeaux, France, [42]Medical Faculty, Institute for Genomic Statistics and Bioinformatics, University of Bonn, Bonn, Germany, [43]University Eye Hospital, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany, [44]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, [45]Department of Zoology University of Lakki Marwat, Khyber Pakhtunkhwa, Pakistan, [46]Center of Functional Genomics, Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Berlin, Germany, [47]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany.

Background: Variants in Brain specific kinase-2 (BRSK2) have recently been associated with a neurodevelopmental disorder (NDD).

Material and Methods: Through international collaboration, we assembled thirty-five novel cases with *BRSK2* variants. We performed negative geotaxis and bang sensitivity assays in *Drosophila* upon knockdown of the fly orthologue *sff* or upon overexpression of human *BRSK2* wildtype and mutant constructs.

Results: Twenty-four individuals carried truncating variants, and eleven harbored missense variants in *BRSK2*, of which seven were located in the kinase domain, one in the UBA domain and two in the C-terminus. Ten variants occurred de novo, twelve were inherited and thirteen of unknown inheritance. Clinical features included variable degrees of developmental delay, normal IQ to moderate intellectual disability and variable neuropsychiatric aspects such as autistic features, ADHD, anxiety or depression.- Upon pan-neuronal knockdown of *sff* in *Drosophila*, we observed impaired locomotor behaviour and increased seizure susceptibility. Furthermore, we investigated the consequences of three missense variants, two of them from the kinase domain. Ubiquitous or pan-neuronal overexpression of human *BRSK2* in flies resulted in lethality or locomotor impairment for the wildtype, respectively, indicating toxicity. The two kinase domain variants had no effect, while the C-terminal missense variant behaved like the wildtype. This indicates a loss-of-function effect at least for the kinase domain missense variants.

Conclusion: Our findings further delineate the clinical and molecular spectrum of *BRSK2*-associated NDD and provide further insights into the role of BRSK2/sff in nervous system function and the specific consequences of missense variants depending on their location.

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P10.006.C Biallelic and monoallelic variants in NCOR2 gene are associated with syndromic neurodevelopemental disorder

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Background: NCOR2 plays a vital role in gene regulation by acting as a transcriptional repressor and modifying chromatin structure through the activation of histone deacetylase 3 (HDAC3). HDAC3 can associate with the related corepressor complex NCoR, and its interaction with these complexes suppresses the transcription of target genes. Recently, a case report described an individual with biallelic *NCOR2* variants presenting with autism and intellectual disability [Prasun, 2024].

Material and Methods: In this study, we report 11 individuals with either biallelic or monoallelic variants in NCOR2 gene. We used the online matching platform (GeneMatcher) to identify individuals with monoallelic and biallelic variants involving NCOR2. Additionally, we conducted functional studies using Drosophila to further investigate the role of Smr, the NCOR2 homolog, in neural development.

Results: Among those individuals, 8 have biallelic variants in NCOR2 and overlapping features, including global development delay, marked speech delay, intellectual disability, autism spectrum disorder, seizures, abnormal tonus, movement disorder, ventriculomegaly and distinctive physical features such as brachycephaly, midface hypoplasia, dysplastic ears, limb and digit anomalies. The remaining 3 individuals with monoallelic variants also present overlapping features including intellectual disability, autism spectrum disorder and movement disorder. We identified nonsense, missense and frameshift variants affecting highly conserved residues. Previous studies in mice highlighted NCOR2 as essential for brain development [Jepsen et al,2007]. Similarly, we observed in Drosophila that Smr (the NCOR2 homolog) is crucial for cell development.

Conclusion: We further define this condition as a multisystem neurodevelopmental disorder resulting from either monoallelic or biallelic variants in NCOR2.

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P10.007.D 32 new cases from Spanish population with intragenic pathogenic variants in *SETD5* gene: refining the phenotype and expanding the genotype

María José Sánchez Soler[1], *María Palomares Bralo*[2], *Fernando Santos Simarro*[3], *María Juliana Ballesta Martínez*[1], *Ana Teresa Serrano Antón*[1], *Vanesa Lopez González*[1], *Carmen María Dolores Sánchez*[1], *Marta Dominguez*[1], *Daniel Doval Calvo*[1], *Irene Valenzuela Palafoll*[4], *Anna María Cueto González*[5], *Encarna Guillén Navarro*[6], *Amaia Lasa Aranzasti*[4], *Veronica Seidel*[7], *Leticia Diana Pias*[8], *Isabel Llano Rivas*[9], *Gracia Eva Li Castan*[10], *Laura Trujillano Lidon*[4]

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Background: Loss-of-function variants in SETD5 gene cause the core phenotype of 3p25.3 microdeletion syndrome characterized by intellectual disability/autism, slow growth, dysmorphic features and malformations such as postaxial polydactyly, congenital heart condition and genitourinary anomalies. The prognosis seems milder in cases with intragenic SETD5 variants.

Material and Methods: Descriptive collaborative study of Spanish children with intragenic variants in *SETD5* gene.

Results:32 cases collected (17 male/15 female), 2 prenatally diagnosed, one inherited. Mean age: 7.5 years (0–38). Prenatal anomalies (14/21): 5 nuchal edema, 4 single umbilical artery, 4 growth retardation and 2 postaxial polydactyly. Postnatally: all exhibited neurodevelopmental disorder, though 3 had normal intelligence. 12/27 hypotonia, 1/31 seizures. Growth: 5/29 short stature, 5/30 microcephaly. Congenital malformations: 10/27 heart disease, 5/26 genitourinary anomalies, 9/26 deafness, 10/24 dental anomalies and 13/21 digital anomalies (6 polydactyly). Main facial features: long/smooth philtrum (81%), arched/thick eyebrows and anteverted/thick nares in 75%. Sequence variants: 5 previously described, 25 nonsense/frameshift, 2 missense and 4 affecting a splice-donor site.

Conclusion:32 new cases and 26 new variants are described expanding the SETD5 phenotype and genotype. 3 cases with normal intelligence support the milder phenotype in these patients. Prenatal anomalies are described in detail for the first time, and two cases were diagnosed prenatally. *SETD5* gene haploinsufficiency should be considered in the differential diagnosis of KBG and Cornelia de Lange syndrome

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P10.008.A Whole exome vs whole genome sequencing in developmental disorders : a nationwide randomized trial

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Background: Whole exome (WES) or whole genome (WGS) sequencing are recommended as first- or second-tier molecular test for the diagnosis of patients with developmental disorders (DD). The clinical utility and cost-effectiveness of WGS however remain debated.

Material and Methods: 566 individuals with an unexplained DD were prospectively recruited across the 8 Belgian human genetics centres and randomized into either the standard of care (SoC) arm, in which WES and a microarray or shallow WGS were applied, or the WGS arm. SNV and CNV calling and interpretation was performed in the diagnostic laboratories with similar softwares and pipelines for both arms. In addition, harmonized reanalysis of the WGS data was performed using the Emedgene variant interpretation platform, integrating STR and SV calling.

Results: WGS had a diagnostic yield of 40.3%, a 10.6% increase compared to SoC (29.7%) ($p = 0083$). This added yield mainly results from a higher detection rate of SNV/indels (+9.1%) with only two pathogenic variants being non-coding. The diagnostic yield was higher in females (46.7%) compared to males (28.1%) ($p < 0.0001$). While de novo variants remain the main identified cause of DD (21.2%), the analysis of inherited variants in genes known to cause autosomal dominant phenotypes lead to more diagnoses (5.2%) than the recessive (2.1%) or X-linked (4.1%) analyses, in the Belgian population.

Conclusion: Our study shows the feasibility of implementing WGS in a clinical setting and demonstrates its growing added value for the diagnosis of patients with DD. Our results also highlight the importance of incorporating inherited variant analysis into routine genetic diagnostics.

C.O.I. Disclosure:

M. Geysens: None. **E. Souche:** None. **H. Van Esch:** None. **K. Van Den Bogaert:** None. **J. Vermeesch:** None.

P10.009.B A novel developmental disorder caused by de novo variants in potassium channel gene*KCNC4*

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Voltage-gated potassium (Kv) channels are critical for neuronal excitability, with mutations in KCNC1-3 linked to neurological disorders. KCNC4 encodes the Kv3.4 potassium subunit and has not been associated with disease. We describe a proband presenting with developmental delay, ataxia, microcephaly, cerebellar atrophy, and hypomyelination alongside a cohort of *KCNC4* variants. Patch clamp electrophysiology revealed three loss-of-function variants (p.Ser268Gly, p.Arg362Cys, p.Leu481Pro) and one gain-of-function variant (p.Arg404His). Computational

modelling of neuronal firing confirmed that these mutations significantly disrupt neuronal excitability. We generated a KCNC4 *Xenopus tropicalis* crispant model and recapitulated patients' phenotypes, including stunted growth, microcephaly and neurodevelopmental defects. Analysis of neuronal firing in the *Xenopus tropicalis* model showed impaired excitability, consistent with the role of Kv3.4 in enabling high-frequency neuronal firing in vivo. These findings establish KCNC4 as a novel disease gene, expanding the spectrum of potassium channelopathies. Our findings have important implications for clinical diagnostics and therapeutic development.

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P10.010.C Biallelic variants in*ITSN1*are implicated in neurodevelopment disorders with severe intellectual disability

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Background: ITSN1 (*Intersectin 1*) is a well-recognized member of the multidomain scaffold ITSN family involved in regulation of endocytosis and play important roles in cell signaling pathways including kinase regulation and Ras activation. Highly expressed in neurons, it is major binding partner for the neuronal GTPase dynamin1. De novo truncating variants in *ITSN1* are implicated in neurodevelopment disorders spectrum. The study summarizes the biallelic variants in *ITSN1* as the genetic basis of neurodevelopment disorders with severe intellectual disability.

Material and Methods: We performed whole exome sequencing of trio and two additional families were identified through GeneMatcher. Disease segregation was confirmed through Sanger sequencing. Molecular biology assays and RNA sequencing was used to investigate the functional impact of biallelic pathogenic variants.

Results: We identified inherited biallelic *ITSN1* truncating (p. Tyr965Ter) and missense variants in three individuals of diverse ancestry who present with microcephaly, global developmental delay, severe intellectual disability, and psychomotor disorder with cortical atrophy. Fibroblasts with truncating variant showed that ITSN1 mRNA transcript is prone to nonsense-mediated decay, and expression of ITSN1 is reduced leading to partial loss of function. RNA sequencing identified metabolic, neurodegeneration and splicing defects pathways implicated in the clinical features of severe intellectual disability and neurodevelopmental disorder.

Conclusion: Our results demonstrate that biallelic *ITSN1* variants are the basis of neurological symptoms through loss of function genetic mechanism mediated through disruption in RNA splicing and neurodegeneration. This is the first human genetics study that links severe ID with disruption in neurodegeneration.

C.O.I. Disclosure:

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P10.011.D Mitochondrial copy number is associated with cognitive function, cognitive decline, and dementia: a longitudinal study in UK Biobank

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Background: Mitochondria is the powerhouse of cell, playing a crucial role in brain health. Mitochondrial copy number (mtCN) is a proxy for mitochondrial function and has been linked to cognitive function and impairment. However, existing studies are often limited by cross-sectional designs, small sample sizes, or a focus on dementia patients.

Methods: Using data from 243,152 UK Biobank participants of European ancestry, we examined the longitudinal relationship between mtCN in blood and cognitive function, as well as its association with cognitive decline, including incident dementia, over a 16-year follow-up. Additionally, we leveraged publicly available genome-wide association study (GWAS) summary statistics to assess the genetic relationship between mtCN and fluid intelligence.

Results: We found that higher mtCN was positively associated with cognitive function. Moreover, higher baseline mtCN was protective against cognitive decline and all-cause dementia over the 16-year follow-up. However, the effects of mtCN observed at population-level differed from those observed in individuals experiencing cognitive decline, suggesting heterogeneous influences of mtCN. Genetic analyses revealed a moderate degree of shared genetic architecture between mtCN and cognitive function, with overlapping genes involved in pathways associated with known mitochondrial diseases. However, no casual effect of mtCN was observed on cognitive function.

Conclusions: Our findings support mtCN as a potential biomarker for cognitive health and cognitive aging. The observed heterogeneity suggests that mtCN may influence cognition through distinct pathways in normal and cognitively declining individuals. Further research is needed to establish the underlying mechanisms.

C.O.I. Disclosure:

C. Xia: None.

P10.012.A Do genomic risk scores contribute to neurodevelopmental and mental health characteristics in CNV-associated intellectual disability?

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Background: Copy number variant (CNV) risk scores are quantitative measures of CNV burden and gene content (loss-of-function intolerance, dosage sensitivity). Population studies have associated CNV risk scores with cognitive and psychiatric traits, however it is not known whether similar patterns exist within CNV-associated Intellectual Disability (ID).

Methods: This study analysed data from the UK-based IMAGINE-ID study. Participants were children and adolescents with neurodevelopmental difficulties and CNVs reported by diagnostic laboratories (N = 1,646, 4–19 years). Multivariate regression models examined the predictive value of CNV risk indices alongside CNV inheritance and socioeconomic status (SES). Outcome measures were developmental quotient, physical disabilities, ASD, ADHD and mental health symptoms from the Development and Well-Being Assessment (DAWBA).

Results: CNV-summated loss-of-function intolerance (pLI) emerged as the strongest predictor of mental health and neurodevelopmental traits, but with unexpected negative associations. Post-hoc analysis identified a threshold effect, whereby lower-range pLI scores (comparable to population samples) predicted higher psychiatric risks, while higher-range pLI scores predicted reduced psychiatric risks. Interpretation of pLI-outcome relationships was complicated by inheritance and SES.

Conclusion: These findings challenge the assumption that higher CNV risk scores universally increase psychiatric risks in children and young people. In the context of CNV-associated ID, genomic risk scores may predict neurodevelopmental and psychiatric outcomes in a complex, non-linear manner. Genomic risk factors interact with inheritance patterns and social deprivation to shape neurodevelopmental and mental health outcomes, highlighting the need for contextualised genetic counselling and support.

C.O.I. Disclosure:

Z. Chi: None. **K.D. Baker:** None.

P10.014.C Switch I variants exhibit selective effects on RAC1

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Background: RAC1-related neurodevelopmental disorder (RAC1-NDD) is characterised by extraordinary phenotypic heterogeneity (e.g. extreme micro- or macrocephaly). RAC1 variants even in the same region of the protein can have opposite functional consequences. The switch I region of this highly conserved small GTPase is critical for effector-binding for activation of downstream pathways, but the functional effects of variants affecting this region have been less explored.

Material and Methods: Identification of switch I variants (via collaborations, variants databases, and literature search), clinical data collection, and fibroblast spreading (for WAVE pathway activity) and RAC1 Raichu biosensor (for PAK binding) assays.

Results: In 22 individuals, we identified 11 distinct pathogenic or likely pathogenic RAC1 switch I missense variants. All individuals showed developmental delay and intellectual disabilities, with the majority also showing microcephaly. Y32H, F37S, and N39D variants exhibited dominant negative (DN) effects on both WAVE and PAK signalling; P34S showed an activating effect on the WAVE pathway but a DN effect on PAK; N39S and S41F showed DN effects on WAVE without significant impact on PAK, while Y40H displayed a DN effect on PAK but had no effect on WAVE.

Conclusion: RAC1 switch I substitutions have variant-specific consequences on downstream signaling pathways. These findings will facilitate diagnosis and provide insights into disease mechanism which are critical for developing treatment strategies.

C.O.I. Disclosure:

H. Althebaiti: None. **S. Banka:** None. **T. Millard:** None.

P10.015.D Identification of a novel recurrent pathogenic *TAF4* variant demonstrates the potential of genomic data sharing in a national healthcare system

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Background: Exome and genome sequencing have greatly improved the diagnostic yield for neurodevelopmental disorders. However, many cases remain undiagnosed due to factors such as unidentified disease genes, and technical and analytical limitations.

Material and Methods: Diagnostic whole genome sequencing was performed by the NHS England Genomic Medicine Service (GMS), with sequencing data processed by the Genomics England bioinformatics pipeline. Variant analysis and interpretation conducted by NHS Genomic Laboratory Hubs, with confirmation by Sanger sequencing.

Results: We identified and confirmed a frameshift variant in a poorly-sequenced repetitive region of *TAF4*, an autosomal dominant intellectual disability gene. Using the Genomics England Clinical Variant Ark, we identified seven further probands (10 individuals in total) heterozygous for this variant, all presenting with a consistent phenotype. Both de novo occurrences and inheritance from affected parents were observed. This variant has not been previously reported, likely due to the repetitive nature of the region interfering with sequencing methods and diagnostic pipelines. However, our findings establish the *TAF4* frameshift variant as a recurrent cause of intellectual disability, and by far the most common pathogenic variant in this gene.

Conclusion: Our work demonstrates the importance of pursuing diagnoses in genomic regions often disregarded due to sequencing challenges; we expect that similar loci may harbour further diagnoses. Furthermore, our findings provide an excellent example of the potential of genomic data sharing within a national healthcare system - through this we were able both to verify our findings and to supply diagnoses to seven additional otherwise-undiagnosed patients.

C.O.I. Disclosure:

M. Spiller: None. **R. Crookes:** None.

P10.016.A Application of highthroughput approaches to investigate the impact of Variants of Uncertain Significance on splicing mechanism in Neurodevelopmental Disorder (NEUDIG project)

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Background: The Massively Parallel Splicing Assay (MaPSy) is employed to evaluate the splicing effects of multiple variants in a single assay. This method uses an amplicon-based library cloned into the pSPL3 plasmid, which is then transfected into HEK293T cells, followed by next-generation sequencing (NGS) analysis. The primary limitation is the lack of a barcode system limiting the total number of variants tested in a single assay. The objective of the study is to Develop high-throughput splicing assays capable of testing up to thousands of variants from Exome Sequencing (ES) data of 1,100-NDD (Neurodevelopmental-Disorder) families (NEUDIG-project). MaPSy as well as a barcode-based approach, named Variant Exon Sequencing (Vex-Seq) have been selected.

Material and Methods: We prioritized 782 ES-identified de novo variants with a SpliceAI score>0.5, MAF < 0.05. 350 had an allele count (AC)<10. Vex-Seq will be used to test wild-type and mutated alleles, in triplicate, totaling 2,100 assays in a unique experiment. Fourteen variants where in SFARI gene database and analysed also by MaPSy assay.

Results: We preliminary tested the SpliceAI prediction on splicing of 14 VUS with MaPSy. For all the fourteen variants (2

exonic, 9 intronic, 2 splicing) aberrant splicing isoforms were detected.The observed aberrant splicing was predicted to generate 11 out-of-frame transcripts, and 4 in-frame transcripts. The Vex-Seq library has been designed and the experiment is ongoing.

Conclusion: Our results confirm that VUS impact on splicing mechanism can be analyzed by a massive approach and SpliceAI is an effective predictor for prioritizing splicing-altering variants.

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P10.017.B Cellular functional tests of *ARX* gene variants provide further insights into a better understanding of genotype-phenotype correlations in both male and female patients

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Background: Intellectual disability (ID) is a major neurodevelopmental challenge, with mutations in the X-linked *ARX* gene being a frequent cause. *ARX*, a master regulator of brain development, encodes a transcription factor orchestrating the delicate symphony of key target genes' expression essential for proper GABAergic neuron development and function. Variants in *ARX* cause a broad clinical spectrum, from asymptomatic female cases to severe neurodevelopmental disorders (NDDs) in male and female patients. This study aims to investigate the functional consequences of 15 *ARX* variants, including likely pathogenic and some novel de novo variants with atypical clinical presentations, such as cases of sudden infant death.

Material and Methods: Missense and frameshift variants were transiently expressed in N2a cells. Functional assays, including Luciferase reporter, Western blot, Immunofluorescence, and RT-qPCR, assessed the level of expression of the produced proteins, their localization, their interaction with known corepressors TLE1 and CtBP1, and their transcriptional activity on a few *ARX*-known targets.

Results: All variants tested exhibited disrupted transcriptional regulation, often linked to altered expression or localization. Interestingly, our findings reveal that some variants exert dominant negative effects, potentially offering an additional explanation for skewed X-inactivation and the unexpectedly severe phenotypes observed in female patients.

Conclusion: Altogether, these findings shed valuable light on the molecular mechanisms driving *ARX*-related NDDs and their remarkable phenotypic diversity. Through a comprehensive review of published *ARX* variants, we aim to establish clear genotype-phenotype correlations based on the nature of the variant (frameshift or missense), its localization, as well as the sex of the patient.

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P10.019.D Genotype-phenotype correlation and familial inheritance of ASXL3-related disorder

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Background: *ASXL3*-related disorder is a neurodevelopmental disorder characterised by developmental delay, intellectual disability, microcephaly, and feeding difficulties. Patients typically harbour de novo heterozygous truncating *ASXL3* variants, which appear to cluster in the first half of exons 11 and 12. This study investigates the correlation between the location or type of variant and disease presentation. We present nine families with inherited *ASXL3*-related disorder and estimate the penetrance of the disorder.

Material and Methods: 204 affected individuals were collated from the literature and a concurrent natural history study. Variants were grouped based on their location and predicted consequence. Phenotypic information was standardised for statistical analysis by Fisher exact or Student t-test, where appropriate. Penetrance was estimated as the proportion of all individuals in published and unpublished families which harbour the familial *ASXL3* variant that present with features of the disorder.

Results: Significantly more individuals with a variant in exon 11 had microcephaly, sleep apnoea, hyperventilation, feeding tube requirement and reflux. Developmental delay and intellectual disability were also more severe in this cohort. Conversely, autism and behavioural problems were more common in the cohort with exon 12 variants. In our families, *ASXL3*-related disorder was inherited from both affected and unaffected parents, with an estimated penetrance of 89%.

Conclusion: These findings suggest a distinction in the disease mechanism of variants in exon 11 and 12, although it is not clear whether variants in exon 11 result in nonsense-mediated decay or truncation. Our pedigrees highlight the variable expressivity and incomplete penetrance of this disorder.

C.O.I. Disclosure:

N. Holmes: None. **E. Woods:** None. **M. Balasubramanian:** None.

P10.020.A Characterization of the role of *PURA* in the neurodevelopmental phenotype associated with 5q31 duplications

Laurine Challeat[1], *Chloé Boisseau*[1], *David Laureanceau*[2], *Solène Remize*[1,2], *Noémie Celton*[2], *Lara Kerbellec*[2], *Céline Pebrel-Richard*[3], *Matthieu Egloff*[4], *Caroline Navarro*[5], *Christine Francannet*[3], *Tanguy Niclass*[6], *Brigitte Gilbert-Dussardier*[4], *Sandrine Vonwill*[2], *Médéric Jeanne*[1,2], *Marie-Laure Vuillaume-Winter*[1,2], *Frédéric Laumonnier*[1,2]

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de Moulins, Moulins, France, [6]Service de génétique, Groupe hospitalier de La Rochelle-Ré-Aunis, La Rochelle, France.

Background: Purine rich element binding protein A (PURA) encoded by the *PURA* gene (5q31) is an important transcriptional regulator that binds DNA and RNA and has a key role in neuronal development and differentiation. Haploinsufficiency of this gene has been reported to cause a severe neurodevelopmental syndrome with a wide range of symptoms including intellectual disability, cerebral abnormalities and seizures. Only one large 5q31 duplication of 3.36 Mb involving this gene has been described by Rosenfeld et al. in 2011 in a patient who exhibits less severe symptoms with global developmental delay. However, the consequences of *PURA* overexpression on neurocognitive development have never been studied.

Material and Methods: Through a national collaboration, we collected seven 5q31 duplications involving *PURA* mainly de novo ranging from 571 kb to 3,5 Mb in patients with a neurodevelopmental disorder. To understand the impact of these duplications on neuronal development, we performed in vitro functional studies on mouse hippocampal neurons as well as human SH-SY5Y neuroblastoma cell line (which can be differentiated into neuron-like cells). *PURA* has been overexpressed in these cells by transfecting a PURA-MYC-DDK plasmid to mimic gene dosage effect.

Results: Overexpression of PURA protein induces a significant decrease in proximal dendritic arborization and in the number of dendrites compared to control condition.

Conclusion: Finally, these in vitro experiments aim to understand the neuropathological mechanisms associated with 5q31 duplications and to determine whether the duplication of *PURA* alters the neurodevelopmental trajectory and induces alterations at the dendritic and synaptic levels.

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P10.021.B MECP2 mRNA profiling in Rett brain showed a mutated monoallelic transcript, while RNAscope analysis in wild type brain defined transcript and cell-specific localization

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Background: Rett syndrome is an X-linked dominant neurodevelopmental disorder mainly affecting girls, caused by MECP2 gene mutations. These include nonsense or frameshift mutations leading to premature protein truncation and mRNA decay, missense mutations in exons 3–4, reducing MeCP2 binding ability to methylated DNA, and deletions that mainly arise in the N-terminal and C-terminal domains. MeCP2 protein regulates gene expression and is highly expressed in the brain, where its dysfunction leads to the disorder neurological manifestations.

Material and Methods: X-chromosome inactivation (XCI), standard histology and MECP2 transcript profile were performed on a post-mortem Rett girl brain specimen carrying the R106W missense variation. This patient belongs to a 54 female subjects' cohort that we have clinically and genetically characterised. Due to specimen quality, further studies were limited. We also defined the MECP2 transcript spatial localization in a control human brain, cerebral cortex and cerebellum areas by RNAscope assay.

Results: The Rett brain specimen showed only the mutated MECP2 transcript, consistent with 95:5 skewed X-chromosome inactivation. RNAscope analysis revealed that MECP2 transcript is highly represented in human adult cerebral cortex and cerebellum, especially in Purkinje and granular cells.

Conclusion: This study confirmed the brain wide MECP2 transcript representation observed with protein analysis. RNAscope provided further insights about the cell-specific and spatial MECP2 transcript localization. XCI analysis in the Rett brain showed that only the mutated MECP2 messenger is transcribed. This finding supports the role of XCI in favouring the mutated MECP2 allele transcription, emphasizing loss-of-function mechanisms and impaired brain early-expressed key genes repression.

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P10.022.C RNU4-2 monoallelic variants as a leading cause of syndromic neurodevelopmental disorder, including in patients with parental consanguinity

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Background: We investigated the role of rare variants in the non-coding RNU4-2 gene as a newly identified cause of neurodevelopmental delay (NDD) and intellectual disability (ID) in a cohort of 4,918 patients with NDD/ID, enriched for parental consanguinity.

Material and Methods: Genome sequencing (GS) data from 22,928 individuals in our Biodatabank were queried for rare, monoallelic variants in RNU4-2. Human Phenotype Ontology (HPO)-encoded clinical information was extracted and analyzed using the ontologyX R package.

Results: Nearly 50% of the 4,918 patients with NDD/ID reported parental consanguinity. Eight relevant heterozygous RNU4-2 variants were identified in 28 patients. HPO-based analysis revealed a consistent syndromic phenotype characterized by NDD, abnormal brain morphology, hypotonia, global developmental delay, microcephaly, seizures, atypical behavior, and facial dysmorphism. RNU4-2 variants accounted for approximately 0.55% of NDD/ID cases in our full cohort, and 0.25% in the subset of consanguineous patients.

Conclusion: This study underscores the significance of *RNU4-2* as a major genetic cause of NDD/ID, extending its relevance to consanguineous patients, where recessive disorders are often suspected. We advocate for the re-evaluation of existing GS data to uncover potential diagnoses and emphasize the importance of GS as a first-tier diagnostic test.

C.O.I. Disclosure:

A. Bertoli-Avella: A. Employment (full or part-time); Modest; CENTOGENE GmbH. **C. Ganoza:** A. Employment (full or part-time); Modest; CENTOGENE GmbH. **M. Ferreira:** A. Employment (full or

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P10.023.D Expanding the mutational spectrum of ReNU Syndrome: insights into 5' Stem-loop variants

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Background: Recent studies have identified pathogenic heterozygous variants in the *RNU4-2* gene, which encodes the small nuclear RNA U4, as the molecular cause underlying ReNU syndrome, a syndromic neurodevelopmental disorder characterized by moderate to severe developmental delay (DD), intellectual disability (ID), a distinct facial gestalt, and multisystem involvement. Causative variants have predominantly been detected within an 18-nt critical region essential for the stability of the U4/U6 snRNA duplex and ensuring the proper spliceosome assembly.

Materials and methods: A cohort of 190 molecularly unexplained DD/ID cases that had previously been found negative via WES/WGS underwent WGS reanalysis or targeted sequencing to identify any pathogenic *RNU4-2* variant.

Results: We report on five affected individuals carrying pathogenic/putative pathogenic *RNU4-2* variants (2.6%). Three individuals harbored the recurrent pathogenic n.64_65insT variant, while two were heterozygous for private/rare maternally inherited variants (n.30A>T, n.43_44insT) within the 5' Stem-loop region. Deep clinical phenotyping reveled a consistent pattern of features, with global DD, ID, brain malformations, and a recognizable facial gestalt. Based on structural homology models and available cryo-EM data, we show that n.30A>T and n.43_44insT are predicted to impair crucial intra-/intermolecular interactions required for spliceosome function.

Conclusion: Our findings broaden the mutational spectrum of ReNU syndrome and confirm the 5' Stem-loop as a second mutational hotspot in *RNU4-2*. We suggest that a more complex genetics likely underlies the inheritance of a subset of *RNU4-2* variants from an apparently unaffected parent, and propose facial gestalt core features as a clinical screening tool to prioritize patients for *RNU4-2* mutation scan.

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P10.026.C Intellectual disability and skin pigmentation anomalies - an unexpected high rate of mosaicism

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Background: Intellectual disability (ID) is a common developmental disorder, either isolated (non-syndromic) or in combination with malformations and abnormalities (syndromic). Despite advances in next-generation sequencing (NGS) technologies like exome sequencing (ES), the molecular cause often remains unresolved, with germline-based diagnostics detecting only about 30% of cases. This highlights the molecular complexity of ID and the need for alternative diagnostic strategies.

Material and Methods: We analyzed blood and tissue samples from 14 patients with ID and associated cutaneous pigmentation disorders (PS). Using exome, genome, RNA sequencing and Nextera deep sequencing, we explored genetic causes, focusing on mosaicism and tissue-specific (fibroblast and melanocyte cultures) mutations in germline-negative cases.

Results: We identified low-grade mosaicism (up to 1.7%) in five patients, involving (likely) disease-causing variants in four known genes (*GNA11, HRAS, HUWE1*, and *OTUD5*) and a novel candidate gene (*SCARA3*). Furthermore, we detected three germline variants in three patients, affecting the genes *TFE3, BNIPL* and *NSD1*. The study achieved an overall detection rate of approximately 60%. These findings highlight the limitations of germline-based diagnostics and emphasize the importance of tissue-specific sampling for precise variant identification.

Conclusion: This study demonstrates that analyzing tissue samples and employing advanced NGS approaches is critical for improving diagnostic yields in ID patients, particularly when germline analysis fails to provide conclusive results. Our results suggest that novel diagnostic strategies, including the assessment of mosaicism and tissue-specific mutations, are essential for uncovering the molecular genetic causes of ID and may provide valuable insights into the pathogenesis of associated syndromic conditions.

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P10.027.D Characterising FBXO11- Associated Intellectual Disability: Novel Features and Genotype-Phenotype Correlations

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Background: Intellectual developmental disorder with dysmorphic facies and behavioural abnormalities (IDDFBA; OMIM:618089) is a recently identified neurodevelopmental syndrome caused by pathogenic variants in *FBXO11*. While its genetic basis is established, the full phenotypic spectrum remains incompletely defined. This study aims to refine the clinical characterisation of FBXO11-related disorder by analysing 16 previously unreported cases.

Material and Methods: Through an international multicentre collaboration, we identified 16 individuals (8 males, 8 females; age range: 17 months to 20 years) with pathogenic or likely pathogenic variants in *FBXO11*. Phenotypic data were collected, and facial dysmorphisms analysed using GestaltMatcher, a machine learning-based tool. Variant classification followed ACMG/AMP guidelines, and genotype-phenotype correlations were explored.

Results: Our cohort exhibited a broad phenotypic spectrum, with mild intellectual disability and speech delay as the most consistent features. Behavioural abnormalities, particularly aggression, were common, while autism spectrum disorder was rare. A characteristic facial gestalt included a broad nasal tip, thin upper lip with a long, smooth philtrum, and large ears. Other features included sleep disturbances, brachydactyly, hyperhidrosis, and joint hyperlaxity. Genotype-phenotype analysis revealed that missense variants were associated with behavioural anomalies, nonsense variants with hypotonia, and large gene disruptions with brachydactyly. Otitis media, previously reported, was not seen in our cohort.

Conclusion: This study refines the phenotypic spectrum of FBXO11-related disorder, highlighting novel features such as hyperhidrosis and brachydactyly while de-emphasizing otitis media. These findings enhance diagnostic accuracy, particularly in mild intellectual disability and behavioural anomalies, where exome sequencing has historically had lower diagnostic yields.

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P10.028.A Fine-scale characterization of the X-linked intellectual disability gene *HCFC1* reveals neuroanatomical phenotypes in female heterozygous mice

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Background: X-Linked Intellectual Disability (XLID) is a neurodevelopmental disorder with X-linked recessive inheritance, characterized by cognitive dysfunction and accounting for about 16% of all cases of ID in males worldwide. However, in most cases, the underlying molecular mechanisms are largely unknown. This project aims to decipher the role of HCFC1, a chromatin-binding transcriptional coregulator, implicated in X-linked cobalamin ID associated with microcephaly.

Material and Methods: Given the full mouse Knockout of HCFC1 is lethal, we utilized the Emx1 Cre line to delete *Hcfc1* specifically in the telencephalon. High-Resolution Episcopic Microscopy (HREM) was used for neuroanatomical studies and 3D Slicer for the segmentation of 24 brain regions, providing

volumetric and morphology data of the entire brain at near-cellular resolution.

Results: Our findings reveal that both male *Emx1Cre;Hcfc1* hemizygote and female *Emx1Cre;Hcfc1* homozygous mice die within hours after birth and present neuroanatomical defects, including complete agenesis of the hippocampus and absence of the corpus callosum. Unexpectedly, we also observed phenotypes in adult heterozygote females, notably a 20% reduction of the volume of both the cortex and the corpus callosum, and a 17% decrease of the hippocampus and the retrohippocampus. The anterior commissure, the fasciculus retroflexus and the fimbria of the hippocampus have smaller volume with 30%, 14% and 10% decrease respectively, in comparison to the matched WT controls.

Conclusion: Our project highlights sex-specific *HCFC1*-associated phenotypes across the mouse brain, suggesting that the medical community assess the contribution of X-inactivation in carrier mothers to help classify variants in probands.

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P10.029.B ARID1B-related disorder in 87 adults: Natural history and self-sustainability

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Background: ARID1B is one of the most frequently mutated genes in intellectual disability cohorts. Thus, far few adult-aged patients with ARID1B-related disorder have been described, which limits our understanding of the disease's natural history and our ability to counsel patients and their families.

Material and Methods: Data on patients aged 18+ years with ARID1B-related disorder were collected through an online questionnaire completed by clinicians and parents.

Results: Eighty-seven adult patients with ARID1B were included. Cognitive functioning ranged from borderline to severe intellectual disability. Patients identified through the genetic workup of their child were either mosaic or had a variant in exon 1. New clinical features identified in this population are loss of skill (16/64, 25%) and recurrent patella luxation (12/45, 32%). Self-sustainability data showed that 88% (45/51) could eat independently, and 16% (7/45) could travel alone by public transport. Facial photo analysis showed that patients' photographs taken at different ages clustered consistently, separate from matched controls.

Conclusion: The ARID1B spectrum is broad, and as patients age, there is a significant shift in the medical aspects requiring attention. To address the changing medical needs with increasing age, we have formulated recommendations to promote timely intervention in an attempt to mitigate disease progression.

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P10.030.C Houge-Janssens syndrome types 1–4: Clinical overview and novel pathogenic mechanisms

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Background: Houge-Janssens syndrome (HJS) is caused by protein phosphatase type 2A (PP2A) dysfunction. PP2A is a trimer of a catalytic (C), a scaffolding (A) and one of 15 regulatory (B) subunits. PP2A oppose the activity of numerous serine/threonine protein kinases, including growth promoting kinases of the PIK3CA/AKT/mTOR and RAS/MAPK pathways. This presentation gives an overview of both the clinical spectrum and known or potential pathogenic mechanisms in Houge-Janssens syndrome.

Material and Methods: All publications on individuals with dominant pathogenic variants in three B subunits (*PPP2R5D* in HJS type 1, *PPP2R5C* in HJS type 4, and *PPP2R5E*), the major scaffolding subunit (*PPP2R1A* in HJS type 2) and the major catalytic subunit (*PPP2RCA* in HJS type 3) were reviewed, clinical features were compared, and genotype-phenotype correlations were investigated and compared to biochemical results on subunit (B to A/C) and substrate (S to PP2A) binding.

Results: We provide a clarifying molecular and clinical comparison of the four types of Houge-Janssens syndrome (HJS) and delineates the molecular mechanisms. This explains why the phenotype can range from learning difficulties and autism to severe intellectual disability with intractable epilepsy. Our study strongly suggests that the regulatory subunits of PP2A are not only important for guiding the phosphatase to the right substrates, but also for protecting these substrates from unwarranted and spontaneous dephosphorylation.

Conclusion: Our work is a reference for understanding clinical and biochemical features of Houge-Janssens syndrome, and our combined results suggest that PP2A has an important role in dephosphorylation timing.

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P10.031.D *DYRK1A* haploinsufficiency syndrome. Clinical and molecular characterization of 33 new cases

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Background: *DYRK1A* haploinsufficiency syndrome (DHS; OMIM#614104; ORPHA:464311;268261) accounts for approximately 1% of cases of intellectual disability (ID) and/or autism spectrum disorder (ASD) and is included in the differential diagnosis of Angelman/Rett-like entities. It is characterized by ID, microcephaly, epilepsy, behavioural abnormalities, and distinctive dysmorphic features.

Material and Methods: We have conducted a retrospective and collaborative study, through the Spanish Society of Clinical Genetics and Dysmorphology, aimed to identify individuals affected by DHS and provide their clinical and molecular characterization, including the biochemical analysis of the associated-kinase activity in new *DYRK1A* variants.

Results and conclusion: A total of 33 cases of DHS were identified across 10 hospitals, involving 25 variants, 15 of which are novel, thereby contributing to an expanded understanding of the molecular basis of the condition. All variants are de novo; moreover, all the missense variants analyzed were catalytically dead supporting functional haploinsufficiency. The main clinical features in the Spanish cohort include ID, epilepsy, autism, microcephaly, and a recognizable craniofacial phenotype, consistent with previously described features, though with lower prevalence of ophthalmological, renal, and feeding anomalies. Unusual features include tibial osteochondroma, bifid uvula, and anteriorly placed anus. Additionally, a patient with macrocephaly and an atypical phenotype, carrying a truncating variant in the C-terminal region that preserves enzymatic activity, may represent a new syndrome associated with *DYRK1A*. The limited number of cases and their concentration in regions with access to medical geneticists suggests that this condition is underdiagnosed, highlighting the need for specialized assessment and comprehensive genomic sequencing studies.

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P10.032.A Refining the Neurodevelopmental Phenotype of Snijders Blok-Campeau Syndrome

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Background/Objectives: Snijders Blok-Campeau syndrome (SBCS) is caused by pathogenic variants (PV) in CHD3. To date, all reported patients have global developmental delay and the majority have mild to severe intellectual disability (ID). However, long-term neurodevelopmental trajectories of affected individuals has been poorly studied to date. This study aims to refine the expected neurodevelopmental prognosis of SBCS.

Methods: We conducted a retrospective analysis of 12 individuals from 10 families, carrying CHD3 PV, identified through WES/WGS, with a median age of 11.5 years [range: 6–42] at last evaluation. Standardized neuropsychological assessments were conducted in 4 patients.

Results: Patients exhibited global developmental delay, with motor delay related to axial hypotonia in all, and language delay in 9/12. Diagnoses of neurodevelopmental disorders could be established or suggested during follow-up: ID ($n = 6$), ADHD ($n = 10$), developmental coordination disorder ($n = 4$), ASD ($n = 3$). Half of our patients attended ordinary classes with human help or classes for children with special needs, the other half attended special schools for children with ID. All individuals of the former group started to speak sentences at or before 3 years of age, whereas those in the latter spoke sentences much later.

Conclusion: This study confirms the broad spectrum of neuropsychological profiles in SBCS. Unexpectedly, half of our patients had a favorable cognitive/schooling outcome, despite universal global developmental delay. Our data suggest that the cognitive performance of children with SBCS may be higher than previously reported, and that early developmental delay does not imply permanent cognitive impairment.

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P10.033.B Expanding the phenotypic spectrum of Helsmorteel-van der Aa Syndrome: a multigenerational case report

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Background: Helsmoortel-van der Aa syndrome (HVDAS) is a rare neurodevelopmental disorder caused by pathogenic variants in ADNP gene, leading to developmental delay, intellectual disability

and autism spectrum disorder. Patients often exhibit characteristic facial dysmorphisms and variable systemic manifestations, including hypotonia and gastrointestinal issues. HVDAS shows a wide range of phenotypic expression, from severe presentations in children to milder forms in adults, with autosomal dominant inheritance.

Material and Methods: An 11-year-old boy was assessed for intellectual disability with speech impairment, recurrent infections and mild dysmorphic features. We performed array-CGH, followed by NGS panel for RASopaties which proved negative. Lastly, whole exome trio sequencing (WES) was performed. Upon identification of a maternally inherited pathogenic variant, targeted segregation analysis in first degree relatives was carried out.

Results: We identified the maternally inherited heterozygous pathogenic variant c.2865_2868del (p.Ser955Argfs*36) in the ADNP gene. Subsequent targeted genetic testing found the variant also in the patient's maternal grandmother and in his sister. Mother (43) and grandmother (74) were both presenting with mild and variable phenotypic features consistent with HVDAS (learning difficulties, mild gastrointestinal symptoms, congenital cataract). His sister (15) was previously assessed for cognitive and language impairment.

Conclusion: This is the first report, to the best of our knowledge, documenting the presence of a pathogenic ADNP variant in elderly individuals, providing new data on the phenotypic spectrum and variability in expressivity. Our findings improve the understanding of HVDAS intrafamilial phenotypic heterogeneity, suggesting that attenuated presentations may occur in adulthood, potentially contributing to the underdiagnosis of this condition.

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P10.034.C Rare genetic variants in neurodevelopmental disorder genes are common in individuals with severe developmental language disorder and normal intelligence

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Background: Developmental language disorder (DLD) is characterized by impaired language development despite adequate linguistic input, and can majorly impact communication and social participation. The role of monogenic causes in DLD is largely unknown and routine genetic testing is rarely offered. This study assesses the diagnostic yield of genome sequencing in severe DLD, and investigates its underlying genetic foundations.

Material and Methods: We designed a prospective cohort study involving trio-based genome sequencing in 25 individuals (aged 3–25y) with severe DLD, normal intelligence (IQ >70) and no autism spectrum disorder. Only individuals with a negative first-degree family history were included, to enrich for de novo variants.

Results: (Likely) pathogenic variants were found in nine individuals, resulting in a molecular diagnostic yield of 36%. The identified genetic causes were diverse, encompassing single nucleotide variants (n = 5), copy number variants (n = 3), and one sex chromosome aneuploidy (n = 1). In seven individuals a de novo variant was found, whilst two had autosomal recessive disorders. The genetic findings provided actionable clinical insights in several cases.

Conclusion: This study shows the value of genome sequencing in individuals with severe language disorders, even in the context of normal intelligence. We observed a high prevalence of rare genetic variants in individuals with severe DLD and a negative family history. Our findings suggest that the molecular background of DLD strongly overlaps with that of other neurodevelopmental disorders. Thus, one should consider (at least a subgroup of) DLD as part of the spectrum of cognitive disorders, rather than a distinct aetiological entity.

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P10.035.D Clinical and molecular characterization of Malan syndrome: new insights from a Spanish case series. Highlighting ophthalmologic manifestations and adulthood care

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Background: Malan syndrome (MALNS) is a neurodevelopmental disorder caused by pathogenic variants in the *NFIX* gene. Patients with MALNS exhibit overgrowth, typical craniofacial features, intellectual disability, and a range of psychobehavioral, musculoskeletal and ophthalmologic signs. The natural history of the disorder is poorly characterized due to the small number of adult individuals described so far.

Material and Methods: We identified 5 unpublished individuals with a neurodevelopmental disorder and overgrowth phenotype, all carrying likely pathogenic variants in the *NFIX* gene. Exome sequencing was used as a diagnostic tool.

Results: The mean age of the individuals is 25 years (range 15–33). Clinical presentation was predominantly characterized by psychobehavioral comorbidities (100%), musculoskeletal involvement (100%), vision impairment (100%), and seizures (50%). Ophthalmologic manifestations were present in all individuals, including strabismus (80%), optic atrophy (40%), and cataract (40%). We report a previously unreported ophthalmologic feature in one patient: unilateral spontaneous absorption of a cataractous lens. Regarding the molecular spectrum, 4 likely pathogenic variants in *NFIX* were identified, none of which have been previously reported. One of the families has two affected daughters, with the variant not identified in the parents, providing evidence of germline mosaicism.

Conclusion: The inclusion of new patients with MALNS increases the understanding of the molecular spectrum of *NFIX* and is essential for delineating the phenotype of this condition. The case series includes a set of sisters with the same novel pathogenic variant in *NFIX*, providing further evidence of germline mosaicism. Ophthalmologic manifestations are prominent and require special surveillance, even in adulthood.

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P10.036.A The GenIDA participatory registry for monogenic forms of neurodevelopmental disorders counts health data files for 2100 affected individuals and allows broad or focused studies of quality of life relevant phenotypes in single gene disorders

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Background: 1700 genes have been reported in genetic forms of intellectual disability or autism, often with limited information on clinical spectrum, comorbidities and natural history. These data are essential for medical care and genetic counseling. We initiated in 2016 an international participatory registry and database to gather such information.

Material and Methods: Clinical information is entered by a family caregiver of an affected individual in a structured questionnaire (41 multiple choice questions and 92 sub-questions, 5 open text qualitative questions) available online in 8 languages (https://genida.unistra.fr). Gene specific charts are generated and updated from the deidentified MCQ data.

Results: GenIDA counts 2095 well documented single patient files, the largest cohorts for KdVS (KANSL1/17q21.31del) 248, Kleefstra syndrome 211, DDX3X 70. The three most represented countries are France (918), USA (399) and UK (176). Comorbidities overlooked previously have been identified such as respiratory infections or childhood asthma in KdVS. The various manifestations of behavior problems or epilepsy and their perceived severity are reported, as well as the perceived efficiency of epilepsy medications. Nine gene specific papers have been published since 2022, reporting broad aspects or more focused studies (scoliosis or ocular manifestations in KdVS, speech and language for DDX3X, arrythmia in Kleefstra syndrome). We will report unpublished observations for MED13L (n = 52) and for the recently identified major ID gene RNU4-2 (n = 23).

Conclusion: GenIDA has proved its utility for investigating the various phenotypes relevant to health, quality of life, cognitive, behavioral and communication aspects in specific neurodevelopmental disorders.

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P10.037.B Expanding the clinical and mutational spectrum of FBXO11-associated Neurodevelopmental Disorder: a monocentric study of 9 additional cases

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Background: FBOX11 encodes an F-box protein involved in the SCF ubiquitin ligase complex, regulating protein turnover. Pathogenic variants in this gene are linked to an autosomal dominant intellectual developmental disorder with dysmorphic facies and behavioral abnormalities (IDDFBA, OMIM:618089). To date,80 cases have been reported, but no germline mosaicism has been described.

Material and Methods: Data were collected from patients with a confirmed heterozygous FBXO11 variant. Genetic testing was performed on DNA extracted from peripheral blood of probands and parents using Clinical Exome Sequencing.

Results: We report 9 individuals from 6 families (age range of 3–48 years). Three were novel variants and one was reported at a low allele fraction of 23% in peripheral blood in a 19-year-old boy. In one family 3 siblings harbored a missense variant, likely inherited from the not tested mother who presented intellectual disability and suggestive facial features. Another family presented two siblings carrying the same variant (both parents tested negative, suggesting germline mosaicism). All patients displayed facial dysmorphism, intellectual disability and/or developmental delay. MRI anomalies (2/9) were described, including ventriculomegaly, hippocampal anomalies, white matter reduction.

Conclusion: This study further molecularly and clinically characterizes FBXO11-associated IDDFBA disorder. We present the first report of a likely germline mosaicism due to the recurrence of an apparent de novo heterozygous variant in two siblings and the first affected individual with allele fraction of 20% in peripheral blood with a full-blown FBXO11-related phenotype. Additionally, the oldest individuals of our cohort help delineate the management recommendations in adulthood.

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P10.038.C Analysis of MeCP2 interaction with the splicing factor PRPF40B and potential implications for Rett Syndrome pathogenesis

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Background: Rett syndrome (RTT, OMIM #312750) is a rare X-linked neurodevelopmental disorder caused by mutations in the *Methyl-CpG-binding protein 2* (*MECP2*) gene in over 95% of cases. MeCP2 is multifunctional protein which regulates gene transcription, chromatin organization, and RNA splicing. Its role in RNA maturation and alternative splicing dysregulation and their contribution to RTT neuropathology remain poorly understood. This study aims to validate and characterize in neuronal cells MeCP2 interaction with PRPF40B, a pre-mRNA processing factor involved in alternative splicing regulation.

Material and Methods: In order to identify differentially expressed and spliced genes associated with MeCP2 mutation, we will compare mutated and isogenic WT iPSC-derived neurons from two patients with the p.R168X mutation through a comprehensive transcriptomic analysis. In parallel, PRPF40B knockdown will be performed in both WT and p.R168X neurons to elucidate its role in MeCP2-mediated splicing regulation. Additionally, co-immunoprecipitation assays are ongoing to validate the interaction between MeCP2 and PRPF40B in human HEK-293 and SH-SY5Y neuroblastoma cell lines.

Results and conclusions: We have demonstrated the binding of MeCP2 to PRPF40B in human HEK-293 and SH-SY5Y neuroblastoma cell lines. By comparing WT and RTT-causing *MECP2* mutant constructs, we have shown that the large C-terminal truncating mutation (p.R168X) abolishes MeCP2 binding to PRPF40B. Validation in patient-derived neurons is ongoing. To further explore the functional significance of the MeCP2/PRPF40B interaction, ribonucleoprotein (RNP) complexes will be isolated, and bound RNA will be characterized. Bioinformatics analyses will identify key genes and pathways associated with the disease, highlighting potential therapeutic targets for RTT.

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P10.039.D Visuospatial abilities predict general adaptive functioning in Williams syndrome

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Background: Williams syndrome (WS) is associated with mild to moderate intellectual disability and specific challenges in visuospatial abilities, characterized by an atypical processing style on tests of construction. To overcome difficulties with visuospatial construction, individuals with WS typically adopt a piecemeal approach resulting in relative success in reduplicating the local elements of an image, but failure to reduplicate global elements. This study ought to address how these specific difficulties with visuospatial construction translate into everyday life in individuals with WS.

Material and Methods: We collected standardized measures of visuospatial ability and adaptive functioning from 24 individuals with WS (50% female, age 9–53 years), and 24 typically developed controls matched on mental age. Regression analyses were used to analyze the data.

Results: The WS group showed severe difficulties with visuospatial construction in comparison to normative data, and applied a piecemeal approach to solve the task. The general adaptive functioning level was low, with a large variation, and characterized by a relative strength in social domains, and challenges with cognitive and practical domains. There was a positive association between visuospatial ability and adaptive functioning, whilst verbal IQ did not add significantly to the model. The final regression model showed that the visuospatial performance predicted 54% of the variation in adaptive functioning scores.

Conclusion: Our findings highlight the importance of clinical assessments and interventions targeting visuospatial difficulties to improve everyday functioning in WS.

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P10.040.A Diagnostic yield in a paediatric cohort of patients with Intellectual Disability, Autism Spectrum Disorder and Epilepsy using a 1568 gene panel and *RNU4-2* sequencing

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Background: For neurodevelopmental disorders (NDDs), a molecular diagnosis is key for management, predicting outcome and counseling.

Material and Methods: Using a custom 1568-gene NGS panel, we analyzed 572 patients (401 males, 171 females) diagnosed with various NDDs, including intellectual disability (ID), autism spectrum disorder (ASD) and epilepsy. All patients had previously negative for array-CGH and FRAXA. A subset of 202 patients was sequenced by Sanger for *RNU4-2*, a spliceosoma RNA component recently implicated in NDDs but not yet included in the panel.

Results: Pathogenic (P) or Likely Pathogenic (LP) variants were identified in 133 cases, yielding an overall detection rate of 23.2%. Among available trios, 54.9% exhibited de novo mutations. Notably, the prevalence of P/LP variants was significantly higher in females than in males (33.9% vs. 18.7%; $p = 0.00008$). Among

patients with epilepsy, 25.6% carried causative variants spanning 23 genes. In those with isolated epilepsy, 20.0% harbored P/LP with *KCNQ2, NALCN, PAK3, PRRT2, SCN1A* and *SLC2A1* being involved in ≥ 2 patients. Among ASD patients, 18% carried P in 11 genes, including *KANSL1, SLC9A6, CDKL5, SYNGAP, SCN1A* and *FOXP1*. No variant was detected in the *RNU4-2* 18-bp critical region, although 2 rare variants were identified in three patients: n.29 A>T (in all the 3 patients vs 5/31388 in gnomAD) and n.96 A>T absent in gnomAD that warrant further investigation.

Conclusion: Our 1,568-gene NGS panel proves to be a powerful diagnostic tool for NDDs in clinical practice and it that will be improved by adding *RNU4-2* and other spliceosome genes.

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P10.041.B Investigating the effect of missense variants in HNRNPU-related neurodevelopmental disorder

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Background: HNRNPU is abundantly expressed in the nucleus where the insoluble portion tethers caRNA to chromatin, and the soluble portion dissociates from chromatin enabling compaction before mitosis. Variants in *HNRNPU* cause HNRNPU-related neurodevelopmental disorder (HNRNPU-NDD). The phenotype of HNRNPU-NDD varies but most patients have intellectual disability, developmental delay, seizures, and craniofacial dysmorphisms. The primary disease mechanism of HNRNPU-NDD is loss of function (LOF), however there is an increasing number of pathogenic missense variants.

Material and Methods: Genotype-phenotype analysis comparing the phenotypes of LOF variant patients and missense variant patients was performed. Site directed mutagenesis was used to create hnRNP-U with the variants of interest and western blotting was done to characterise the impact of four missense variants in the ATP binding domain of the protein.

Results: No phenotype difference was seen between missense variant patients and LOF variant patients. Functional analysis showed that proteins carrying missense variants are still expressed and hence not targeted for degradation. It also showed that missense variants in the ATP binding domain of hnRNP-U reduce protein solubility. The result of this is the protein is only able to tether caRNA to chromatin, it is unable to evict the RNAs in time for mitosis.

Conclusion: A similar phenotype for both LOF and missense variants suggest there is a similar disease mechanism hence, missense variants must be causing a qualitative or quantitative LOF. Functional analysis shows that this is likely to be a qualitative LOF with the four variants studied decreasing the solubility of the protein.

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P10.042.C Functional evidence supports expansion of the CEP104 disease spectrum beyond Joubert Syndrome

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Background: Ciliopathies are a group of congenital disorders caused by cilia dysfunction. Cilia are small signaling organelles present on many eukaryotic cells that play an important role during development. The ciliary protein CEP104 is required for cilium elongation and sonic hedgehog signaling (SHH). Biallelic pathogenic variants in *CEP104* cause Joubert syndrome (JBTS), characterized by psychomotor developmental delay, oculomotor apraxia and cerebellar hypoplasia visualized as a 'molar tooth sign' (MTS) on brain imaging. More recently, variants in *CEP104* were also associated with intellectual disability disorder 77, a non-syndromic neurodevelopmental disorder without the characteristic MTS. We describe a young girl with biallelic variants in *CEP104*, who presents with a neurodevelopmental disorder phenotype without MTS. To investigate the pathogenicity of the variants we studied ciliogenesis and SHH in proband-derived fibroblasts.

Methods: Skin-derived fibroblasts of the proband and three controls were evaluated by immunofluorescence for ciliogenesis, ciliary length, microtubule stability, and SHH properties. Cilium parameters were quantified using the automated FIJI-tool ALPACA. The effect of the missense variant on protein-protein interactions was assessed by co-immunoprecipitation.

Results: CEP104 proband-derived fibroblasts displayed slightly shorter cilia and a significant difference in SHH component levels in comparison to controls, while ciliogenesis and microtubule stability were not affected. The missense variant also disturbed CEP104 binding to CSPP1.

Conclusion: Our results suggest pathogenicity of the *CEP104* variants and thereby provide a clinical and molecular diagnosis for this family. Furthermore, it provides evidence to expand the *CEP104*-associated disease spectrum beyond JBTS and suggests to investigate *CEP104* in patients with intellectual deficit.

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P10.043.D *SETD5*-related neurodevelopmental disorder: 50 novel individuals and review of the literature

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Background: Mendelian disorders of the epigenetic machinery are a broad group of neurodevelopmental disorders involving various components of the epigenetic machinery and other functionally related genes. *SETD5* gene (OMIM *615743) maps to chromosome 3 (3p25.3), and encodes for the lysine methyltransferase family member SET Domain Containing 5. The primary role of SETD5 is promoting gene expression through the trimethylation of lysine 36 of histone H3 (H3K36), a crucial event for chromatin partitioning. Pathogenic variants in *SETD5* are associated with a neurodevelopmental disorder known as "intellectual developmental disorder, autosomal dominant 23" (OMIM #615761, ORPHA: 404440) featuring intellectual disability of various degree, hypotonia, feeding difficulties, dysmorphic features, autism spectrum disorder, and behavioral disorders.Up to know about 35 patients have been described in literature in several reports.

Material and Methods: we launched an international call via the ERN ITHACA website to improve the current knowledge of this condition and collected 50 novel cases.

Results: we present our clinical data comparing them with what already known in literature.

Conclusion: our work has allowed us to broaden the phenotypic description of the *SETD5*-related neurodevelopmental disorder, also confirming the role of ERN-ITHACA as valuable platform for data exchange

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P10.044.A Neurodevelopmental profile of Kleefstra syndrome: nature and trajectory of regressions

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Kleefstra syndrome is a rare genetic disorder caused by haploinsufficiency of the EHMT1 gene: microdeletion in chromosomal region 9q34.3 (in 85% of cases) or mutation in the EHMT1 gene coding for euchromatin histone methyltransferase 1, characterized by the core clinical phenotype of intellectual disability, hypotonia, severe speech delay, and distinct facial characteristics with additional clinical features including sleep disturbances with an erratic sleep pattern, overweight, psychiatric disorders, and autism spectrum disorders. Among psychiatric manifestations, anxiety, mood disorders, psychosis have been reported as an enduring severe apathy (Verhoeven et al, 2010). Regressive behavioral phenotype might appear at adolescent/adult age (Willemsen, 2012).We report five young adults including two twin sisters who develop psychiatric catatonia-like manifestations associated with regression. Therapeutic care has shown lack of response to traditional treatments for catatonia and strategy was based on anti-psychotic medications. For one case diagnosis of dysimmun encephalitis was evoked with improvement after encephalitis management and anti-psychotic medications.We also report five patients with late onset psycho-motor regression along with somatic manifestations as major weight loss, signal abnormalities on brain MRI and unfavorable evolution.As for some others neurodevelopmental disorders, poor evolution in adulthood tends to be described in Kleefstra syndrom as illustrated by cases presented, suggesting existence of a neurodevelopmental neurodegenerative continuum.

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P10.045.B Wieacker-Wolff-Syndrom (WRWF): From a novel variant of uncertain significance to a likely pathogenic variant in *ZC4H2* using RNA sequencing

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Background: WRWF is a severe X-linked neurodevelopmental delay disorder caused by deleterious variants in *ZC4H2*, encoding a zinc-finger protein involved in interneuron differentiation. Affected males show fetal muscle weakness, contractures, delayed motor development, facial and bulbar weakness, and dysmorphic facial features. We present a patient with a novel *ZC4H2* variant, which was reclassified as likely pathogenic (LP) following cDNA analyses.

Material and Methods: Trio whole-exome sequencing (WES); targeted cDNA analyses.

Results: An 8-months-old boy with severe congenital dystonia, severe developmental delay, hypotonia, and dysmorphic facial features was referred to our outpatient clinic. By trio exome sequencing, an intronic hemizygous variant of uncertain significance (VUS) was detected in the index patient: NM_001178032.3(ZC4H2):c.156+5G>T r.spl? p.? (PM2_SUP). His asymptomatic mother was a heterozygous carrier. After three years, reassessment of the variant revealed a patient carrying a G>A-substitution at the same position. This variant resulted in aberrant splicing using a cryptic donor splice site at c.156+5 (PMID 26056227) which led to the insertion of 45 bp into the mRNA. We conducted cDNA analyses, demonstrated a similar aberrant splicing event in the family presented herein and reclassified the variant as likely pathogenic (PM1, PM2_SUP, PM4, PP3).

Conclusion: By integrating cDNA sequencing, we demonstrated aberrant splicing and, in theory, in-frame insertion of 15 amino acids. Our report highlights the importance of comprehensive genetic and transcriptomic (re)analyses. This underscores the need for functional validation of VUS to improve the accuracy of variant interpretation, curation, and patient management.

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P10.046.C Molecular diagnosis of neurodevelopmental disorders: insights from 300 cases

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Background: Neurodevelopmental disorders (NDDs) are a group of heterogeneous conditions affecting 5−10% of people worldwide and the leading cause for referral genetic clinical services. Despite significant advances in genomic technologies, achieving a molecular diagnosis remains challenging due to their complex genetic architecture, requiring multiple testing approaches.

Patients and Methods: We investigated 300 patients affected by NDDs using a multi-tiered molecular analysis approach combining SNP−array and NGS. Initially, all patients underwent SNP-array analysis. Subsequently, negative cases underwent 338 genes panel sequencing involved in neurodevelopmental disorder.

Results: Our integrated approach achieved a molecular diagnosis in 30% of cases (91/300). In details, we found pathogenic or likely pathogenic CNVs in 15% (46/300) of cases involving critical chromosomal regions, including 1p36, 2p16.3, 7q11.23, 15q11-q13, 16p11.2, 22q11.21, and Xp22. TRS analysis allowed us to reveal a causal or likely causal SNVs in 18% (45/254) of cases. Among them, of particular interest a de novo microdeletion affecting *BICRA* a gene recently associated to Coffin−Siris syndrome type 12, a splice−altering synonymous variant in *KMT2E* validated through RNA analysis, a novel missense variant in *ADNP*'s DNA−binding domain for which bioinformatics analysis revealed its destabilizing effect on protein structure.

Conclusion: Our multi−modal approach achieved a diagnostic yield comparable to current literature and revealed multiple novel disease−causing variants. Future studies integrating multi−omics approaches and periodic reanalysis of negative cases may improve diagnostic yield and maybe uncover new NDD genes.

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P10.047.D Modeling Schaaf-Yang Syndrome: Insights from iPSC-Derived Neurons

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Background: Truncating mutations in *MAGEL2* are associated with Schaaf-Yang syndrome (#MIM 6615547; SYS), a severe ultra-rare neurodevelopmental disease. SYS patients present with intellectual disability, neonatal hypotonia, sleep disorders, and joint contractures, among other symptoms. MAGEL2 contributes to the regulation of protein recycling pathways, circadian rhythm, and mRNA metabolism.

Material and Methods: Using transcription factor-mediated differentiation, 4 SYS and 4 control iPSC lines were differentiated into iNeurons (iNs) and iAstrocytes (iAs), and co-cultured. Proteomic and transcriptomic analyses were carried out to identify expression alterations at the mRNA or protein levels. Additionally, calcium imaging was performed on forebrain neurons differentiated from SYS and control neural progenitor cells (NPCs) to assess neuronal activity over time and along differentiation.

Results: iNs and iAs were successfully generated from SYS and control iPSCs. Integrated proteomics and transcriptomics analyses revealed distinct molecular signatures, highlighting dysregulations in extracellular matrix organization and collagen degradation pathways. NPC-derived cultures changed upon development, exhibiting gradually more synchronous activity patterns. Calcium imaging in forebrain neurons uncovered functional traits of SYS physiopathology.

Conclusion: The obtained iNs and iAs establish a relevant in vitro model for SYS. The identified DEGs in SYS iNeurons suggest a potential novel role for MAGEL2 in extracellular matrix organization, and could serve as biomarkers for monitoring future therapeutic strategies. These findings lay the groundwork for further investigations into MAGEL2's role in neuronal activity and function.

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P10.048.A Variants in *SNRPN* underlie a variable neurodevelopmental disorder

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Background: Small nuclear ribonucleoprotein polypeptide N (*SNRPN*, MIM*182279), a bicistronic imprinted paternally expressed gene, is located in the Prader-Willi critical region (PWCR) on chromosome 15q11.2-q13. Till date, two single-nucleotide variants in *SNRPN* have been identified in two affected individuals from two unrelated families with Prader-Willi syndrome (PWS).

Material and Methods: Detailed clinical evaluation followed by exome sequencing was performed in the probands. Allele-specific RT-PCR was performed to determine the parental origin of the mutated allele. In vivo functional studies using *Drosophila melanogaster* was carried out to determine the developmental and behavioral phenotypes of the identified variants.

Results: Proband 1 presented with speech delay, mild intellectual disability (ID), seizures, and truncal obesity and harbored a de novo heterozygous missense variant, c.260C>A p.(Pro87His) in *SNRPN* (NM_003097.6) on his paternal allele. Proband 2, with clinical features overlapping with PWS was identified with a paternally expressed variant, c.280C>T p.(Arg94Trp) in *SNRPN* inherited from her asymptomatic father.

Functional studies show that knockdown of *SmB*, fly homologue of *SNRPN*, during early development leads to larval lethality with impaired locomotion and early developmental defects. We show that the variants identified in the study, c.260C>A and c.280C>T are evolutionary conserved and confirm them as loss-of-function alleles using rescue experiments in *Drosophila*. Additionally, knockdown of *SmB* in mature neurons led to increased inter-male aggression, increased food intake in males, and increased daytime sleep in isolated females.

Conclusion: We herein validate the association of variants in *SNRPN* with a neurodevelopmental disorder with clinical spectrum ranging from isolated ID to PWS.

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P10.049.B A*de novo**KCNA3*and an inherited*KCNQ3*variants associated with reduced potassium channels expression in a patient with developmental and epileptic encephalopathy, intellectual disability, and behavioral anomalies

Enrica Marchionni[1], *Vito Luigi Colona*[2,3], *Emanuele Agolini*[4], *Michela Murdocca*[3], *Monia Russo*[3], *Maria Stellato*[3,5], *Valeria Latini*[3,5], *Anna Maria Nardone*[1], *Paola Spitalieri*[3], *Luigi Mazzone*[5], *Antonio Novelli*[4], *Federica Carla Sangiuolo*[1,3], *Giuseppe Novelli*[1,3]

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Background: In 2024, de novo missense *KCNA3* variants were linked to a developmental and epileptic encephalopathy characterized by marked speech delay, motor delay, intellectual disability (ID), and autism spectrum disorder (ASD)[1]. We report a 14-years-old boy presenting with drug-resistant epilepsy, marked hetero-aggressiveness, psychomotor agitation, hyperkinesis, CC -2SD, and ID (Leiter-3 QI:41). A de novo heterozygous NM_002232.5(KCNA3):c.1433T>A, p.Val478Glu variant and a heterozygous maternally inherited NM_004519.3(KCNQ3):c.1720C>T, p.Pro574Ser variant were detected through a targeted NGS panel and trio-exome sequencing. The aim of this study was to characterize the effect of these variants.

Material and Methods: Human dermal fibroblasts (HDFs) were isolated from the patient and an age-matched control' skin biopsy.*KCNQ3/KCNA3* mRNA expression was analyzed by quantitative RT-PCR, while KCNQ3/KCNA3 protein expression was analyzed by Western Blot (WB) and quantified by densitometric analysis.

Results: Compared to the control, transcript levels of *KCNQ*3 and *KCNA3* markers in patient-HDFs were reduced by 40% and 77% respectively (***$p = 0.0002$; ****$p < 0.0001$). WB confirmed a statistically significant reduction in KCNQ3 and KCNA3 protein levels (35% and 50%, respectively; *$P < 0.05$) in patient-HDFs.

Conclusion: The *KCNA3*-related phenotype aligns with the proband's clinical features. However, the same *KCNQ3* variant has been reported in three unrelated individuals with isolated ASD; in two cases, it was inherited from an asymptomatic parent[2]. To date, patients carrying both *KCNA3/KCNQ3* variants have not been reported. The reduced expression of both proteins in patient-HDFs suggests the contributing role of the rare de novo *KCNA3* variant with the inherited *KCNQ3* one, possibly acting as a modifier of the patient's complex phenotype.[1]PMID:37964487 [2]PMID:23596459

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P10.050.C A study of six Turkish patients with Alazami syndrome: clinical and genetic insights

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Background: Alazami syndrome (ALAZS) is a rare disorder caused by biallelic loss-of-function variants in the *LARP7* gene. It presents with developmental delays, intellectual disabilities, primordial dwarfism, and distinct facial dysmorphism. To date, the literature has documented approximately 55 cases.

Material and Methods: This study presents six patients from five families who were evaluated and molecularly diagnosed with ALAZS by various centers across Türkiye. Furthermore, we provide a comprehensive review of the existing literature on previously documented cases.

Results: A cohort of six patients, consisting of four males and two females, had an age range of 22 months to 15 years at the examination. All patients exhibited characteristic features of ALAZS, such as intellectual disability (ID), developmental delay, and reduced height. Antenatal manifestations, including IUGR, were noted in all of the patients ($n = 6$, 100%). Other findings included microcephaly ($n = 6$, 100%), gait imbalance ($n = 3$, 50%), genitourinary issues ($n = 4$, 66%), gastrointestinal manifestations($n = 3$, 50%) and cardiac abnormalities ($n = 3$, 50%). Molecular analysis revealed five distinct variants across five families, comprising five frameshift variants.Additionally, one out of the six patients had a dual diagnosis, having ichthyosis vulgaris.

Conclusion: This study provided more insights into ALAZS's phenotype and genotype, integrating our findings with a literature review to define and emphasize its characteristics more clearly.

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P10.052.A Expansion of *MAD1L1*-associated phenotype: second case with chromosomal aneuploidy

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Background: Loss-of-function (LoF) variants in *MAD1L1* have been recently described as the cause of autosomal recessive disorder characterized by chromosomal aneuploidy, systemic inflammation and increased tumor predisposition in a single case: an adult

woman with multiple benign and malignant neoplasms, dysmorphic features, short stature and microcephaly.

Material and Methods: A teenage boy with intellectual disability (ID), short stature, dysmorphic features, microcephaly and epilepsy was referred to our diagnostic center for whole exome sequencing (WES) and chromosome analysis.

Results: Duo-WES analysis identified two rare, heterozygous LoF variants: c.1396C>T p.(Gln466*), inherited from mother, and c.820_823delGAGA p.(Glu274Profs*52) in *MAD1L1*. In addition, WES identified a previously reported rare heterozygous LoF variant in *CACNA1A* c.4869+1G>T r.spl p.?, which provides a genetic cause of the ID and epilepsy, but also indicates incomplete penetrance as the variant was inherited from mother. The contribution of these three variants together to the patient`s phenotype currently remains unclear. In addition, chromosome analysis in boy´s lymphocytes revealed mosaic aneuploidy in 36% of analyzed metaphases.

Conclusion: We report the second case with two LoF variants in *MAD1L1*, providing further evidence that the loss of MAD1L1 causes an autosomal recessive syndrome with chromosomal aneuploidy. Both known cases present short stature, microcephaly and dysmorphic features. Despite comparable levels of aneuploidy, our case has not shown so far evidence of tumorigenesis or inflammation, indicating possible variability in phenotypic expression but also highlighting the need for surveillance. Additional cases and functional studies are needed to better define the clinical spectrum of *MAD1L1*-associated aneuploidy syndrome.

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P10.053.B Somatic mosaicism of unmethylated FMR1 full mutation and heterogeneous premutation alleles in an asymptomatic older male

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Background: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism, caused by full mutations (FM) of the *FMR1* gene with expansions of over 200 CGG repeats in the 5'UTR region. These expansions lead to promoter methylation, resulting in transcriptional silencing and loss of the FMRP protein. Premutations (PM), with 55–200 CGG repeats, remain transcriptionally active but cause toxic overexpression, leading to premutation-associated conditions (FXPAC). Rare cases of FXS with little or no methylation show residual gene function, associated with better cognitive outcomes. Complex patterns of methylation and mosaicism increase the risk of FXPAC. Objective: A thorough clinical evaluation was performed on a 77-year-old asymptomatic male, who was found to have a complex and rare molecular mosaicism, with both FM and a wide range of premutation alleles, all unmethylated.

Methods: Clinical assessment included neurological exams, IQ tests (WAIS-IV, MMSE), cognitive evaluations, gait analysis, and sEMG. Brain MRI checked for degeneration. RNAseq analyzed FMR1 gene function.

Results: Neuropsychological evaluation revealed normal or higher-than-normal performance in several cognitive domains, with balance disturbances and abnormalities in posture, ground reaction forces, muscle activity, and joint angles. No other neurological signs were observed. Preliminary RNAseq data showed increased FMR1 mRNA levels and identified differentially expressed genes involved in neuronal function, synaptic plasticity, and cellular stress response.

Conclusion: Investigating these rare cases could reveal genetic and functional mechanisms that protect against clinical manifestations, providing important insights into factors that modulate the wide phenotypic variability in both FXS and FXPAC.

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P10.054.C Genetic causative aberrations and diagnostic yield in children with Autism spectrum disorders

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Background: Autism spectrum disorders (ASD) are considered a complex disorder, with a high heritability of up to 80%. However, only 10–20% of ASD cases can be diagnosed as single-gene pathogenic variants or chromosomal aberrations. The male-to-female ratio is approximately 3–4:1, and comorbidity (seizures, mental retardation) occurs in approximatelyy 50% of ASD cases.

Material and Methods: To determine the presence of causative genetic variants in a cohort of 84 children with ASD, we performed the following analyses: G-band karyotyping, FMR1 gene (CGG expansion), MECP2 gene, whole-genome microarray, and whole-exome analyses, including trio-exome in some patients.

Results: The male-to-female ratio in study group was 5:1. The overall diagnostic yield of genetic causes in ASD patients was 12%. No chromosomal abnormalities were found by karyotype analyses. Genetic analyses on the *FMR1* and *MECP2* genes confirmed fragile X syndrome in five boys and Rett syndrome in one girl with ASD (8.7% of those tested). In three boys with ASD, a de novo single gene causative variant (*TBL1XR1, NAA15, PRKAR1B*) was found, and in one boy, a deletion of the 2q23.3q24.2 region (16% and7% of those tested, respectively). Seven pair of siblings were present in our cohort (17% of the study group), but no genetic causative variant was found among them.

Conclusion: Our study highlights the importance of genetic testing as an attempt to incorporate genetic causes into a comprehensive causal framework of ASD, which may help clinicians to provide tailored counseling and clinical action in children with ASD, given the 12% diagnostic yield.

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P10.056.A Description of two patients with novel biallelic variants in *GTF3C3* and associated phenotype review

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Background: Biallelic variants in *GTF3C3* have recently been associated with an autosomal recessive form of syndromic intellectual disability. To date, only 12 affected individuals have been reported. We present two previously unreported patients carrying novel biallelic variants in *GTF3C3* and a review of the phenotype associated in the patients described to date.

Material and Methods: Two patients were selected for the study. Patient 1 is a 22 years-old Spanish female who was subjected to Whole-exome sequencing and further Sanger sequencing for segregation studies. Patient 2 is a Pakistani 22 years-old female who was studied by trio-whole exome sequencing. Variants were classified using the American College of Medical Genetics and Genomics (ACMG) guidelines. The clinical features of these patients were combined with data from previously reported cases to provide a comprehensive overview of the disorder.

Results: Genetic analysis identified compound heterozygous variants c.2498A>G and c.1708C>T in Patient 1, confirmed to be in trans, and a homozygous missense variant c.266T>A in Patient 2. Both patients presented with intellectual disability and generalized epilepsy as the core symptoms. By integrating these findings with data from previously reported cases, the frequency of all observed manifestations of the syndrome was quantified.

Conclusion: This study contributes to a more comprehensive understanding of *GTF3C3*-related syndromic intellectual disability by adding two newly identified patients with novel biallelic variants.

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P10.057.B Expanding the Genotypic Landscape of TBL1XR1-Related Conditions: A Novel Variant in Pierpont Syndrome

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Background: Pathogenic variants in the TBL1XR1 gene are associated with Pierpont syndrome (PRPTS; MIM #602342), a rare autosomal dominant disorder characterized by global developmental delay, axial hypotonia, specific craniofacial dysmorphisms, and abnormal subcutaneous fat distribution. The condition is commonly associated with the c.1337A>G (p.Tyr446Cys) variant and, more rarely, other missense variants. These variants cluster in the WD40 repeat regions of the protein, crucial for protein-protein interactions. Recent studies implicate TBL1XR1 in a broader neurodevelopmental spectrum, including intellectual disability and autism spectrum disorder, with or without dysmorphic features.

Material and Methods: We report a 4-year-old male with global developmental delay, microcephaly, hypotonia, autistic-like behaviors, and distinctive facial features. Additional findings included brachydactyly and fetal pads. Neonatal neuroimaging revealed porencephaly and interhemispheric asymmetry. Genetic testing included karyotyping, FMR1 CGG repeat analysis, SNP-array, and exome sequencing.

Results: Exome sequencing identified a novel heterozygous NM_024665.7: c.959_961del, p.(Asn320del) in-frame deletion in TBL1XR1, absent in the maternal DNA. Paternal DNA was unavailable to confirm de novo status. This variant is absent in population databases. Functional predictions indicate a pathogenic effect, supported by the high conservation score of the affected residue across vertebrate species and its location within a mutational hotspot in the WD40 repeat regions. According to ACMG criteria, it was classified as likely pathogenic.

Conclusion: Our findings expand the genotypic landscape of TBL1XR1-related conditions, highlighting the role of in-frame deletions in Pierpont syndrome pathogenesis. They underscore the need for comprehensive genotype-phenotype correlation studies to refine diagnostic and therapeutic strategies for this rare yet heterogeneous disorder.

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P10.058.C Biallelic variant, c.2511C>G p.(Ser837Arg) in *CNTROB* is associated with a neurodevelopmental disorder

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Background: Centrobin, encoded by *CNTROB*, is a core component of the centrosome and is asymmetrically localised to daughter centrioles during mitosis. A biallelic canonical splice-site variant c.355+1G>A in *CNTROB* has been recently reported in a 14-year-old female with intellectual disability, short stature, microcephaly, cataract, abnormal teeth, obesity, hypertension, and insulin resistance. Centrobin-depleted zebrafish embryos have shown microcephaly, curved and shorter bodies, and morphological features indicating ciliary dysfunction.

Material and Methods: A detailed clinical evaluation followed by exome sequencing (ES) was performed on the proband after obtaining informed consent.

Results: A one-year and seven-month-old female presented with developmental delay and failure to thrive. Antenatally, oligohydramnios and intrauterine growth retardation were documented, and she was born with a low birth weight (−1.85SD). She has microcephaly (−3.6 SD), short stature (−3.1 SD), and is underweight (−3.3 SD). Examination revealed facial dysmorphism, generalised hypotonia and an ejection systolic murmur. Magnetic resonance imaging of the brain showed white matter volume loss and a thin corpus callosum. Echocardiogram showed ostium secundum-type atrial septal defect. ES revealed a biallelic missense variant c.2511C>G p.(Ser837Arg) in *CNTROB* (NM_053051.5). In silico tools [SpliceAI - 0.48 (donor loss); 0.26 (acceptor loss) and Human Splice Finder Pro] predict the variant to cause aberrant splicing. Splice-site analysis using reverse transcription PCR from RNA extracted from skin fibroblasts is being processed.

Conclusion: We herein report a family with a biallelic variant in *CNTROB* associated with a neurodevelopmental disorder. Further validation of this variant and collaborative efforts are necessary to validate the gene-disease association.

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P10.059.D An iPSC model for the SRCAP-related neurodevelopmental disorder "Floating-Harbor Syndrome"

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Background: Floating-Harbor-Syndrome (FLHS) is a rare disease, characterized by intellectual disability, short stature and facial dysmorphism. FLHS is caused by heterozygous nonsense mutations in the FLHS locus of the SRCAP gene. SRCAP plays a versatile role in different cellular functions including chromatin remodeling, gene regulation, DNA repair and cell division.

Material and Methods: Induced pluripotent stem cells (iPSCs) from a healthy donor were modified using CRISPR/Cas9 to introduce a mutation in the FLHS locus. Unspecific mutations in off-target regions were excluded by Sanger sequencing and chromosomal integrity was confirmed using low coverage whole genome sequencing. Expression of pluripotency factors and germ layer differentiation was examined with RT-qPCR and immunofluorescence microscopy.

Results: The modified cells showed a heterozygous deletion in the FLHS locus, resulting in a premature stop codon previously described in FLHS patients. No unspecific modifications or chromosomal abnormalities were detected in these cell lines. The cells tested positive for pluripotency markers OCT4, SOX2, NANOG, TRA1-60 and were able to differentiate into cells belonging to all three germ layers.

Conclusion: The cell lines presented here fulfill the criteria to be considered pluripotent and both carry the same pathogenic mutation in the FLHS locus. These two cell lines can therefore serve as iPSC models to investigate the function of SRCAP in the context of various cell types, including e.g. cortical neurons. This will allow further investigations into the pathogenic effects of SRCAP mutations on a molecular and cellular level and thus gain insights into the etiology of FLHS.

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P10.060.A De novo missense variant in DNA-binding homeobox domain of ADNP causes Helsmoortel-van der Aa syndrome in a 24-year-old woman

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Background: Helsmoortel-van der Aa syndrome (HVDAS) is a rare neurodevelopmental disorder typically caused by truncating variants in ADNP, which encodes for a 1,102-amino-acid protein arranged into multiple functional domains. To date only 3 missense variants has been described in medical literature, and all carrier patients seems to show milder phenotypes compared to truncating variants.

Patient and Methods: We describe a 24-year-old Italian girl with intellectual disability (HP:0001256), speech and language delay (HP:0000750), microcephaly (HP:0000252), truncal obesity (HP:0001956), elevated serum testosterone levels (HP: 0030088), increased circulating dehydroepiandrosterone sulphate concentration (HP:0034589), aggression towards others (HP:5200125) and self-injurious behaviour (HP:0100716), amenorrhea (HP:0000141) and polyphagia (HP:0002591). DNA of the patient underwent a multistep molecular analysis. Structural modeling of wild-type and mutated DNA binding domain of ADNP was obtained using Alphafold 3, while protein-DNA binding affinity was conducted by using PDA-Pred tool.

Results: Targeted resequencing identified a novel heterozygous de novo ADNP missense variant c.2405C>T, p.(Ser802Phe) in the DNA-binding homeobox domain. MetaDome analysis revealed that this variant is located in a region that is highly intolerant to missense variants. Structural modeling showed that an introduction of an aromatic amino acid may alter intramolecular interactions and binding with DNA. Protein-DNA binding affinity showed that wild-type conformation is energetically favorited.

Conclusion: We describe the first missense mutation affecting the ADNP DNA-binding homeobox domain. Our findings suggest that this variant may impairs DNA interaction and gene expression regulation. Our adult patient, who is the oldest reported to date, sheds light on the long-term progression of HVDAS.

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P10.062.C Expanding the phenotypic spectrum of *PAN2*-related neurodevelopmental disorder: two siblings with a novel homozygous frameshift variant

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Background: Biallelic, truncating variants in *PAN2*, encoding a mRNA deadenylation complex subunit, have been reported in six affected individuals from four families with a syndromic neurodevelopmental disorder (NDD) including variable intellectual disability, hypotonia, sensorineural hearing loss, EEG abnormalities, multiple congenital anomalies, short stature, skeletal anomalies, and facial dysmorphism.

Methods: Diagnostic quattro-exome sequencing was performed in two siblings with NDD and their consanguineous, healthy parents.

Results: A novel homozygous frameshift variant (c.1661del p.(Phe554Serfs*42)) was identified in both siblings. Parents were heterozygous carriers. The 13 year old girl presented with severe intellectual disability, MRI anomalies (multiple, nonspecific white matter lesions subcortical and periventricular, hypoplasia of the vermis with slight rotation), febrile seizures, unilateral cataract,

combined moderate hearing loss, and short stature with reduced IGF-1 and normal IGFBP-3 levels. Due to elevated gonadotropins, ovarian insufficiency was suspected. Her 6 year old brother had severe intellectual disability, epilepsy, sensorineural hearing loss, and brain malformations such as polymicrogyria, malrotation of the hippocampus and focal cortical dysplasia. In both siblings, facial dysmorphism were noted including prominent supraorbital arches and eyebrows, short palpebral fissures, a long philtrum, a thin upper lip, and slightly low-set, backward-rotated ears. Furthermore, broad thumbs, clinodactyly fingers V, and hypoplastic fingernails were noted.

Conclusion: While the siblings showed overlapping clinical findings with the affected individuals reported in literature, severe brain malformations such as in the boy were not described before. Our findings therefore contribute to a further characterization of the phenotypic spectrum of the *PAN2*-related neurodevelopmental and multiple congenital anomalies disorder.

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P10.063.D Unusual clinical presentation in a girl with a novel de novo loss-of-function (LoF) variant in *FRYL* identified through Whole Exome Sequencing (WES) reanalysis

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Background: Reanalysis of WES data has proven an effective strategy for diagnosing neurodevelopmental disorders or malformations after inconclusive initial results. However, variant interpretation remains challenging, particularly for highly penetrant ones such as LoF variants, due to complex genotype-phenotype correlations. Recent studies identified de novo LoF and missense variants in *FRYL* in 14 patients with developmental delay, intellectual disability (ID), congenital anomalies and dysmorphic features.

Material and Methods: WES and data reanalysis; clinical follow-up.

Results: We report the case of a 7-year-old girl referred to our department with a syndromic condition. She was born at term by cesarean section, after a pregnancy complicated by polyhydramnios. At birth, she presented with severe respiratory distress and hypotonia. MRI revealed corpus callosum hypoplasia and tetraventricular hydrocephalus. Cognitive milestones were achieved (current IQ = 112), but she had lower limb hyposthenia and hyporeflexia and never gained the ability to walk. EMG/ENG studies and muscle biopsy were normal. She also has severe scoliosis, mild unilateral renal hypoplasia, and tricuspid regurgitation. Reanalysis of WES data identified a de novo c.3944delA, p.(Asn1315Thrfs*7) variant in *FRYL*. This frameshift variant is predicted to be pathogenic and is likely to result in a premature stop codon, with subsequent degradation of the transcript by nonsense-mediated decay (NMD). In vitro studies on patient-derived fibroblasts are underway to confirm this hypothesis.

Conclusion: Our patient is the first reported without ID but with peripheral neurological involvement and inability to walk, expanding the clinical spectrum of FRYL-associated disorders and underlying the importance of systematic data reanalysis.

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P10.064.A Genetic diagnostics of neurodevelomental disorders using targeted gene panel sequencing

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Background: Neurodevelopmental disorders (NDDs) represent a diverse group of conditions that affect the development and function of the brain. Identifying their genetic causes is crucial for prognosis, reproductive counseling, and family support. This study assessed the diagnostic yield of gene panel sequencing in patients who underwent targeted gene panel sequencing due to diagnoses of intellectual disability, global developmental delay, or autism spectrum disorder.

Material and Methods: We retrospectively analysed 605 patients (417 males, 188 females) referred between 2019 and 2023. All had negative results from karyotyping, arrayCGH, and fragile X syndrome testing before gene panel sequencing. The number of sequenced genes, enrichment method and sequencing platform varied over the years. Initially, 270 samples were analysed using a targeted Custom Intellectual Disability panel from SOPHiA (286 genes) and sequenced using the MiSEQ platform (Illumina). The next 335 samples were enriched using a clinical exome solution from SOPHiA (539–602 genes) and sequenced on NovaSeq (Illumina). Variants were prioritised and interpreted using SOPHiA DDM software, based on frequency, predicted pathogenicity, and the ACMG guidelines. Where applicable, variants were validated using Sanger sequencing.

Results: The diagnostic yield was 15.2% (92/605), with most cases involving autosomal dominant de novo variants. Patients with severe ID, MRI abnormalities or hearth defects were more likely to have a positive genetic result.

Conclusion: This study highlights the effectiveness of targeted gene panels in diagnosing ID/GDD and aligns with previous findings[1,2,3], emphasizing its role in clinical practice.

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P10.065.B Detection of X-inactivation profiling through RNA sequencing

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Background: Despite advances in DNA-based diagnostic techniques, a portion of neurodevelopmental disorders (NDDs) remains undiagnosed. X-chromosome inactivation (XCI) can be leveraged to identify missing heritability. To explore this, we aim to integrate RNA sequencing (RNA-seq) and XCI profiling with DNA sequencing.

Material and Methods: We studied a preliminary cohort of 7 females: six exhibiting XCI-skewing (>80%) — comprising three mothers of NDD patients, three NDD cases — and one NDD case with a random XCI, by methylation-sensitive STR-profiling. All tested negative for CMA, FRAXA and exome (ES). We integrated RNA-seq with exome sequencing data to identify allelic imbalance.

Results: In the six females with skewed XCI, RNA-seq confirmed a chromosome-wide allelic imbalance, showing that the allelic expression of X-linked heterozygous variants deviated from the expected range for random inactivation (observed ratio: 0.01–0.26, which is lower than the expected 0.5 ± 0.15, previously reported [PMID: 30552425]). Conversely, in the female with random XCI, RNA-seq validated a balanced allelic ratio (observed ratio: 0.40). Further analyses are underway to identify which of the mono-allelically expressed genes could be causative for the NDD.

Conclusion: RNA-seq combined with XCI profiling presents a promising approach for investigating undiagnosed NDD cases. This method has the potentially to enhance diagnostic yield by identifying functional and epigenetic abnormalities that lead to XCI imbalance.

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P10.066.C De novo missense variants in *FBXW11* associated with diverse neurodevelopmental phenotypes: insights from two new cases

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Background: The *FBXW11* gene, encoding an F-box protein involved in ubiquitination, has been implicated in neurodevelopmental disorders. We present two new cases with *FBXW11* (NM_001378974.1) missense variants, highlighting phenotypic variability and its correlation with variant location.

Material and Methods: Patients: Case 1: A 2-year-old girl with a de novo variant: c.1399G>T (p.Val467Phe) in the F-box domain. Phenotype includes global developmental delay, microcephaly, hypotonia, and dysmorphic features. **Case 2:** A 6-year-old boy with a de novo variant: c.1319G>C (p.Gly440Ala) in the WD40 repeat domain. Phenotype includes autism spectrum disorder (ASD), ADHD, and social communication difficulties. Brain imaging was normal.

Methods: Whole-exome sequencing identified *FBXW11* variants. Both were classified as likely pathogenic (ACMG Class 4), absent in gnomAD (v4.1.0) and in-house databases. Variants are located in critical domains: F-box (Case 1, exon 5) and WD40 repeat (Case 2, exon 10).

Results: The girl's variant is associated with severe developmental delay and structural anomalies, consistent with prior reports linking the F-box domain to brain malformations. The boy's variant correlates with behavioral and cognitive impairments, such as ASD and ADHD, aligning with WD40 repeat domain involvement in autism-related phenotypes.

Conclusion: These cases suggest a genotype-phenotype correlation in *FBXW11*-related disorders: F-box domain variants are linked to severe developmental delay, while WD40 repeat domain variants are associated with behavioral and cognitive impairments. This highlights the importance of variant location in predicting clinical outcomes and expands understanding of *FBXW11*'s role in neurodevelopmental disorders.

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P11 Neurogenetic and Psychiatric Disorders

P11.001.D Long range enhancer-promoter interaction maps contribute to understanding the impact of non-coding de novo variants on autism spectrum disorder risk

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Background: Whole-genome sequencing (WGS) studies of autism spectrum disorder (ASD) have started to highlight the role of rare de novo variants (DNVs) within non-coding regulatory regions. However, it is still difficult to predict their functional consequences. We recently performed RNApolIII-ChIA-PET of mouse brain-derived neural stem cells (mNSC), which identified a list of thousands of human-mouse syntenic Long Range Interactions (hmsLRI), connecting gene promoters to (often very distant) enhancers. These regions are expected to be functionally relevant for neurodevelopment, representing a valuable resource for variant interpretation in ASD.

Material and Methods: We analyzed WGS data of our cohort of 105 ASD families and three large ASD collections, to identify DNVs overlapping with hmsLRI regions. We developed mNSC transgenic for defective Cas9-KRAB, to direct epigenetic repression to specific enhancers (or, as a control, the connected gene promoter transcription start site, TSS), via suitable guideRNAs, to monitor the effect on the expression of the connected gene via qRT-PCR.

Results: We showed that evolutionarily conserved DNVs in hmsLRI regions are enriched in ASD probands ($p = 0.0141$) and we identified the genes functionally connected to the enhancers carrying conserved DNVs. Focussing on recurrently mutated elements, we started the experimental validation of the effect of selected DNVs. We obtained 90% downregulation of the expression of *FOXG1* by guide-RNAs directed against the TSS, and are now testing the comparative effect of repressing connected enhancers.

Conclusion: These results highlight the significance of long-range interactions in the potential impact of conserved DNVs within regulatory regions on specific target genes.

C.O.I. Disclosure:

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P11.002.A Whole genome sequencing of 1500 Parkinson's disease patients of an Italian cohort

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Background: Parkinson's disease (PD) is heterogeneous both clinically and genetically with complex phenotypes and different progressive courses. In addition to recent advancements on the identification of both rare and common variants contributing to disease onset and risk, a comprehensive evaluation of the whole genome landscape in subjects with PD will improve our knowledge of the genomic basis of this disease. The study aims at deciphering the genetic impact of coding and non-coding regions of genomes of PD patients in Northern Italy and at integrating whole genome sequencing (WGS) in clinical practice.

Material and Methods: WGS is conducted on PD subjects with short-read (Illumina) technology. Additionally, for selected cases, long-read (Oxford Nanopore) sequencing is also performed. Bioinformatic analysis is carried out using the GPUs-accelerated NVIDIA Clara™ Parabricks® automatized pipeline. We also developed a consensus pipeline for Structural Variants (SVs) analyses.

Results: We have carried out WGS of approximatively 1500 PD patients on the Illumina platform and long-read sequencing on a selected group of subjects. In a subset of cases, we have identified pathogenic and risk variants in known causative genes. We have also identified Copy Number Variants that haven't been detected with standard clinical methods.

Conclusion: The integration of short- and long-read sequencing allows the identification of novel mutations and mechanisms/pathways in unsolved clinical cases with PD. Overall, this study demonstrates that incorporating WGS in clinical practice allows the detection of diagnostic variants that conventional methods failed to identify.

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P11.003.B *CAPRIN1* haploinsufficient cortical organoids helps in identifying underlying neurodevelopmental defects (Award Candidate)

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Background: CAPRIN1 is a ubiquitously expressed protein involved in essential cellular processes, including proliferation and cell migration. In brain, it is recruited to neuronal RNA granules and directly modulates transport and translation of mRNAs that are necessary for synaptic plasticity. Haploinsufficiency of CAPRIN1 has been implicated in neurodevelopmental disorders.

Material and Methods: Human induced pluripotent stem cell (hiPSC) of three *CAPRIN1*-patients and three healthy controls were used to generate forebrain cortical organoids (fBOs). Metabolic analysis was performed using Seahorse mitochondrial stress tests. Single-cell RNA sequencing is in progress.

Results: Morphological analysis of *CAPRIN1*-patients derived organoids show defects in growth and proliferation, suggesting an altered composition of the forebrain organoids. An overrepresentation of immature neurons and glial cells was observed using flow cytometry. Since these cells predominantly rely on glycolysis, β-oxidation, and redox signalling for their metabolic processes, we conducted metabolic analyses. Preliminary results suggest an increase in glycolysis and a rise in reactive oxygen species (ROS). Single cell-RNA sequencing has also been performed at different stages of fBO maturation, to dissect their cellular composition and analysis of the results is ongoing.

Conclusion: Following our previous work (PMID: 35979925), our results suggest that *CAPRIN1* haploinsufficiency impacts the cellular fate and composition of neuronal compartments, giving initial explanation to the neurodevelopmental defect observed in patients. The 3D cortical organoid model enables the

identification of cellular and metabolic mechanisms that could serve as therapeutic targets for this disorder.

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P11.004.C Dynamic X-chromosomal reactivation enhances female brain resilience

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Neurodevelopmental disorders exhibit an intriguing sex bias with females being less often and less severely affected than males. While neurodevelopmental disorders-associated genes are evenly distributed across all autosomes, they are overrepresented on the X-chromosome highlighting the particularly important role of the X-chromosome in the phenotype formation of Neurodevelopmental disorders. In males, most X-linked genes are hemizygous, i.e., males only carry the allele inherited from the mother. In contrast, random XCI in female cells results in a mosaic expression of X-linked genes in female tissue, substantially influencing phenotype development of X-linked gene defects. However, hemizygosity of X-linked genes in males and random XCI in females only partially explain the fact that females are disproportionally less affected by neurodevelopmental disorders. By leveraging the clonal status of human iPSCs with a skewed XCI, we established an in vitro system of neurodevelopment for the detection of facultative escape genes during neuronal differentiation trajectories. By analysing transcriptomic data at allele-specific resolution, we identified two novel categories of facultative escapees that change their expression from the inactive X-chromosome depending on the cell´s differentiation status and specific cell type. The dynamic reactivation of facultative escapees substantially influenced the phenotype of brain organoids generated from a patient with an X-linked neurodevelopmental disorder, revealing an unknown layer of female brain protection. Taken together, our data demonstrate the dynamic nature of a set of genes located on the inactive X-chromosome, able to change their expression profile in a temporal- and lineage-specific manner.

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P11.005.D Interplay of polygenic scores and diverse environmental factors in psychiatric disorders (Award Candidate)

Tahereh Gholipourshahraki[1], *Linda Ejlskov*[2], *Birgitte Dige Semark*[1], *Genona Torruella Maseras*[1], *Jette Steinbach*[1], *Ole Sahlholdt Hansen*[1], *Carsten Bøcker Pedersen*[1], *John Joseph McGrath*[1,3,4], *Michael Eriksen Benros*[5,6], *Preben Bo Mortensen*[1], *Liselotte Vogdrup Petersen*[1], *Esben Agerbo*[1], *Bjarni Jóhann Vilhjálmsson*[1,7,8]

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Background: Psychiatric disorders represent a significant global health burden with complex etiologies involving both genetic and environment. While the independent effects of these factors have been thoroughly investigated, their interactions in shaping psychiatric disorder risk remain poorly understood. This study aims to investigate the interaction between Polygenic scores (PGS) as proxies for genetic risk and environmental factors at both the individual and family levels for psychiatric disorder risk.

Material and Methods: This study utilized the iPSYCH case-cohort sample (n = 141,265). Environmental factors, including region, urbanicity, parental socioeconomic status and history of inflammatory and infectious diseases, were obtained from Danish nationwide registers. Generalized logistic regression was used to examine the effects of PGS, environmental factors and their interactions on psychiatric disorders. Analyses were adjusted for age, sex and (PCA). Inverse probability weighting was applied to account for selection bias.

Results: Both PGS and environmental factors were associated with psychiatric disorders, consistent with previous findings. We found evidence of gene-environmental interactions across several psychiatric disorders. For instance, individuals with a history of autoimmune disease showed a stronger association between PGS and bipolar disorder. Those with an early-life brain injury (≤10 years) showed a stronger PGS association for schizophrenia. The most consistent finding emerged in the analysis of parental education, which moderated the PGS-diagnosis association across all psychiatric diagnoses investigated.

Conclusion: We found evidence that some environmental factors may moderate genetic susceptibility to psychiatric disorders.

Additional studies in diverse populations are needed to validate these findings and enhance applicability across different settings.

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P11.006.A Polygenic burden of neurotransmitter and ion channel pathways in generalzied and focal epilepsy (Award Candidate)

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Background: Common genetic variants contribute to epilepsy risk. We investigated the polygenic burden and pathway-specific polygenic risk scores (PRS) in neurotransmitter (GABAergic, glutamatergic) and ion channels pathways across genetic generalized epilepsy (GGE), focal epilepsy (FE), and GGE subtypes. This study leveraged data from the ILAE and the Epi25 Consortiums.

Material and Methods: GWAS summary statistics were used to construct pathway-specific PRS for European-descent individuals with GGE (n = 3,305) and FE (n = 9,095), compared to 24,218 controls. GGE subtypes included juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE), generalized tonic-clonic seizures (GTCS), and childhood absence epilepsy (CAE). Associations were assessed using logistic regression.

Results: GABAergic PRS showed significant enrichment in GGE (OR = 1.15–1.20, $P < 0.05$) but modest enrichment in FE (OR = 1.00–1.05, $P > 0.05$) across multiple databases (GO, Reactome, KEGG). Within GGE subtypes, JME showed the strongest GABAergic associations. Glutamatergic pathway PRS demonstrated elevated significant burden in GGE (OR = 1.15–1.20, $P < 0.05$) and modest in FE (OR = 1.00–1.05, $P > 0.05$), with notable enrichment in JME. Ion channel analysis revealed divergent effects for calcium channels, showing significant positive association in GGE (OR = 1.08–1.12, $P < 0.05$) but negative association in FE (OR = 0.92–0.98, $P < 0.05$), while voltage-gated cation channels showed consistent positive associations across both epilepsy types.

Conclusion: This study highlights differential polygenic influences on neurotransmitter and ion channel pathways in GGE, FE, and GGE subtypes, particularly opposing calcium channel effects. Distinct GABAergic and glutamatergic patterns were observed in GGE, especially in JME and CAE, providing insights of polygenic burden in epilepsy.

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P11.007.B Genome-wide analysis of tandem repeat expansions in Autism Spectrum Disorder

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Background: Tandem repeat expansions (TREs) are associated with over 60 monogenic disorders. Recent whole genome sequencing (WGS) studies highlighted their involvement in autism spectrum disorder (ASD), but further studies are necessary to better elucidate the role of specific TREs in ASD risk.

Material and Methods: We analyzed WGS data of 105 ASD families using ExpansionHunter Denovo (EHdn), to identify TREs whose lengths were outliers compared with other members of the cohort. TREs were filtered according to Z-score>10 and TR length>150 bp (high-confidence, HC-TREs). Then we used ExpansionHunter to accurately genotype the most interesting TREs in our cohort and in 629 European individuals from the 1000 Genomes Project WGS data, and REViewer to generate read visualizations for all individuals at each locus. The frequency of specific TREs was assessed using TR-gnomAD data. Experimental validation was performed using fluorescent Long-Range PCR (LR-PCR) and Repeat-primed PCR.

Results: We identified 280 HC-TREs from 124 ASD individuals. We then prioritized 10 TRE in genes already implicated in ASD or other neurodevelopmental disorders (NDD). Among these, the most interesting one is a rare AAG expansion in the intron 11 of *CACNB1*, a gene recently highlighted among the top candidate ASD-relevant tandem repeat loci. Interestingly, LR-PCR confirmed that the tandem repeat, expanded in the unaffected mother, is further expanded in the ASD proband.

Conclusion: The identification of tandem repeat expansions in *CACNB1* and other ASD/NDD risk genes strengthens the role of rare TRE in the genetic aetiology and phenotypic complexity of ASD.

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P11.009.D Severe recessive *ADCY5* related encephalopathy: a case report and literature review

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Background: *ADCY5*-related disorder (*ADCY5*-RD) is primarily an autosomal dominant movement disorder characterized by paroxysmal exacerbations during sleep or awakening, occasionally associated with neurodevelopmental delay, with or without intellectual development disorder. Most of the associated variants are missense, with a presumed gain-of-function mechanism.

Only four families with recessive *ADCY5*-RD have been reported to date, with a more frequent neurodevelopmental component, often severe and harboring missense, splicing, or frameshift variants. We present the 5th family with a recessive form of *ADCY5*-RD.

Material and Methods: Two cases from a consanguineous family were explored for a syndromic movement disorder by exome sequencing through standard care.

Results: Exome sequencing identified a novel splice site variant, NM_183357.3: c.2898_2900+22del p.(?) in the homozygous state, inherited from asymptomatic heterozygous parents. Their phenotype was reminiscent of the one described in previously reported

recessive ADCY5-RD cases with a severe neurodevelopmental outcome, abnormal movements, feeding difficulties, failure to thrive, and premature death for some cases.

Conclusion: We hypothesize a loss-of-function mechanism in recessive *ADCY5*-RD, with a presumed correlation between phenotype and residual ADCY5 activity. Additionally, we highlight rare dominant *ADCY5*-RD cases for which the gain-of-function mechanism was questioned.

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P11.010.A Brain-restricted chromosome 1q gains underlie focal epilepsy with hyaline astrocytic inclusions

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Background: Brain somatic mosaicism is an emerging cause of neurological disorders, including cortical malformations and epilepsy. Here, we aimed to identify the genetic basis of focal epilepsy with hyaline astrocytic inclusions, a distinct neuropathological entity.

Methods: We analyzed matched blood and surgically-resected brain tissue from eight children with drug-resistant focal epilepsy and hyaline astrocytic inclusions using deep whole exome sequencing, genome-wide copy number analysis, and fluorescence in situ hybridization (FISH). We performed laser capture microdissection of astrocytes followed by targeted sequencing, and multi-electrode array (MEA) recordings to assess epileptiform activity.

Results: We identified brain-specific mosaic chromosome 1q gains in 6/8 patients that were absent from blood. Variant allele analysis revealed these gains originated from maternal meiosis, suggesting they were subsequently lost from non-neural lineages through postzygotic rescue. The 1q gain was significantly enriched in astrocytes with hyaline inclusions. Local epileptiform activity were investigated for correlation with the density of affected astrocytes in acute cortical slices.

Conclusion: We establish chromosome 1q gain as a genetic cause of focal epilepsy with hyaline astrocytic inclusions and show that brain-restricted mosaicism can arise through postzygotic rescue of meiotic errors. Our findings highlight tissue-specific mosaicism as a novel disease mechanism and suggest astrocyte-specific chr1q gains contribute to epileptogenesis in this distinct clinicopathological entity

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P11.011.B Integration of proteomics enables the establishment of genetic diagnoses in a cohort of dystonia affected individuals (Award Candidate)

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Background: Dystonia is a movement disorder characterized by vast genetic and phenotypic heterogeneity. Most patients are left without conclusive genetic diagnosis following whole exome (WES) and whole genome sequencing (WGS). However, the added benefit of proteomic analyses of patient-derived material in this disease category has not been systematically explored yet.

Material and Methods: Primary fibroblast cell lines derived from 76 dystonia index cases and four affected relatives were subjected to bulk proteomics and the expression profiles were analyzed using PROTRIDER tool (1). Complementary RNA sequencing was done for a subset of selected cases and the output was inspected with IGV. Short-read WGS data was available for all study participants.

Results: The analysis of proteomic expression changes enabled the verification of WGS-detected variants in genomic regions prone to sequencing and alignment errors (*IRF2BPL* and *MECP2*) and the re-classification of one variant of uncertain significance to likely pathogenic (*SLC16A2*). Furthermore, proteomic outlier detection highlighted pathogenic gene underexpression in three cases (*SPG11*, *UFC1*, *ATM*) caused by (deep) intronic variants. Transcriptomic analysis corroborated the deleterious effect of these intronic variants on splicing integrity.

Conclusion: Systematic implementation of proteomics in the dystonia cohort provided key information for 8.6% (6/70) WGS-inconclusive index patients. Adding omics layers such as proteomics further supports genomic variant interpretation and increases the diagnostic yield in dystonia patients with ambiguous WES and WGS findings.

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P11.012.C *ADGRB2*,*ERF*, and*WDR5*as Novel Candidate Genes for Dystonia based on High-Throughput Exome Sequencing

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Background: Dystonia is a neurological movement disorder with high clinical and genetic heterogeneity. Despite substantial

Table 1. Presumably pathogenic variants in novel dystonia candidate genes

Gene	Genomic variant (hg38)	cDNA variant	Protein variant	gnomAD v4.1.0 European	CADD v1.7	Sex	Age at onset	Dystonia type	Additional features	Family history
ADGRB2	chr1-31742112-C-T	c.1358G>A	p.Trp453*	not found	38	f	38	Cervical	none	negative
ADGRB2	chr1-31742838-C-T	c.1252G>A (splice site)	p.His364_Val418del (predicted)	not found	34	f	50	Blepherospasm + laryngeal	none	negative
ADGRB2	chr1-31744258-TA-T	c.1021del	p.Tyr341Metfs*83	1.7e-6	35	f	35	Cervical	none	negative
ERF	chr19-42249498-C-CG	c.613dup	p.Arg205Profs*24	not found	39	m	9	Cervical, cranial, upper limbs	turricephaly	negative, de novo
ERF	chr19-42249415-G-A	c.697C>T	p.Arg233*	not found	28.3	m	30	Generalized (cervical, cranial, trunk)	cranial flattening (high fronto-parietal)	negative
WDR5	chr9-134141543-A-G	c.224A>G	p.Tyr75Cys	3.4e-6	24.6	m	24	Cervical	none	n.a.
WDR5	chr9-134141961-G-A	c.277G>A	p.Val93Ile	5.4e-5	24	f	39	Cervical	none	negative
WDR5	chr9-134141971-C-T	c.287C>T	p.Ser96Leu	8.5e-7	34	m	23	Cervical	none	negative

n.a.=information not available.

heritability (>25%) and >400 dystonia-linked genes, the etiology remains unresolved in most patients, suggesting pathogenic variants in additional genes contribute to the disorder.

Methods: Exome sequencing data from 1,924 genetically unsolved dystonia patients, including five trios, was searched for variants in genes not yet linked to dystonia. Variants were filtered based on frequency in controls, in silico predictions, gene constraint, segregation, and literature on gene function. Genes with multiple hits were prioritized. An independent cohort of 1,825 patients from Munich, Germany, was analyzed to identify additional variant carriers.

Results: We identified *ADGRB2*, *ERF*, and *WDR5* as novel dystonia candidate genes. In *ADGRB2*, a G-protein coupled receptor critical for neurodevelopment, two truncating and one splice site variant were identified in our sample (Table); patients shared a late-onset cervical/cranial dystonia phenotype. For *WDR5*, involved in histone modification and an interactor of the dystonia gene *KMT2B*, three missense variants were associated with adult-onset cervical dystonia. In *ERF*, encoding a transcription factor, we identified a de novo frameshift variant, and an additional stop-gain variant was found in the Munich cohort; patients had predominantly upper body dystonia (Table).

Conclusion: Our findings suggest that variants in *ADGRB2*, *ERF*, and *WDR5* may contribute to dystonia, supported by high-impact variants, similar phenotypic patterns, and biological functions consistent with known dystonia genes.

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P11.013.D CAG repeats in *HTT*, *ATXN1* and *ATXN2* genes as modifiers of the phenotype in mental disorders: schizophrenia and bipolar disorder. (Award Candidate)

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Background: Intermediate alleles (IAs) of short tandem repeats (STRs) in *HTT*, *ATXN1*, and *ATXN2* genes have been linked to neurodegenerative and psychiatric disorders. Moreover, *HTT* IAs have been associated with bipolar disorder (BD), suggesting a genetic influence on BD phenotype. Huntington's disease (HD) is characterised by motor, cognitive, and psychiatric manifestations, which may lead to clinical overlap with BD. Schizophrenia-like psychosis occurs in HD in 6–25% of cases, with the paranoid form being the most common. Additionally, early-onset HD has been associated with a greater risk of developing psychosis, further highlighting the shared genetic and clinical features between these conditions. These findings underscore the importance of genetic screening to improve the diagnosis and management of neuropsychiatric disorders.

Material and Methods: This study analysed *HTT*, *ATXN1*, and *ATXN2* CAG repeats in 334 BD, 234 SCH patients and 1070 controls. Statistical analyses included Chi-squared, Fisher's Exact, Kruskal-Wallis with Dunn's post hoc, and regression models.

Results: A higher *HTT* IAs frequency in BD (7.4%) compared to controls (4.1%) was found, but the adjusted p-value (0.067) suggests only a trend. *ATXN1* IAs frequency was higher in BD (12.5%) than in controls (7.2%) ($p = 0.0598$) and significantly higher compared to SCH (4.7%) ($p = 0.03$). *ATXN2* IAs showed no significant differences across groups. Additionally, two expanded alleles in BD for *HTT* (0.9%) and one in SCH for *ATXN2* (0.5%) were identified.

Conclusion: These results suggest that *HTT* and *ATXN1* IAs may contribute to BD susceptibility, reinforcing the genetic overlap between neuropsychiatric and neurodegenerative disorders.

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P11.014.A Expression of miR-185 during neural differentiation of induced pluripotent stem cells from patients with 22q11.2 Deletion Syndrome

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Background: The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans and it is associated with high rates of neurodevelopmental disorders (NDDs). Literature data revealed downregulation of miR-185 expression, located in the 22q11.2 region, in the whole blood of patients with 22q11.2DS. Here we analyze miR-185 expression during neural differentiation of induced pluripotent stem cells (iPSCs), derived from patients with 22q11.2DS, and search for miR-185 potential target genes associated with NDDs.

Material and Methods: The expression of miR-185 was assessed by Taqman qRT-PCR using specified Taqman assay for miR-185. miRNet platform was used to elucidate miR-185 function in NDDs. miR-185 target genes were predicted by using miRDB, mirWalk and TargetScan. The selection of miR-185 target genes related to NDDs was conducted using online available tools GeneTrack, miRNet and Metascape.

Results: Decreased expression of miR-185 was detected in iPSCs derived from patients with 22q11.2DS, compared to healthy control. In addition, the expression of miR-185 was significantly downregulated in neurons from patients with 22q11.2DS compared to expression detected in neurons of healthy control. Using online available tools, we selected three potential target genes of this miRNAs related to NDDs - *CSMD2*, *DLG2* and *HIC2*.

Conclusion: Obtained results indicate that miR-185 and it target genes *CSMD2*, *DLG2* and *HIC2* could serve as novel therapeutic targets. Future studies are needed to evaluate their relationship and involvement in neurodevelopmental outcome seen in patients with 22q11.2DS.

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P11.015.B Repeat expansions with small TTTCA insertions in *MARCHF6* cause Familial Myoclonus without Epilepsy

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Background: Familial Adult Myoclonus Epilepsy (FAME) is a rare autosomal dominant disorder caused by the same intronic TTTTA/TTTCA repeat expansion in seven distinct genes. TTTTA-only expansions are benign, while those containing TTTCA insertions are pathogenic. We investigated the genetic basis of dominant cortical myoclonus without seizures in two unrelated families.

Material and Methods: Repeat-primed PCR, long-range PCR and nanopore sequencing were used to detect and characterize expansions at known FAME loci.

Results: We identified a novel repeat expansion in *MARCHF6*, comprising elongated TTTTA repeats (388–454) and 5–11 TTTCA repeats at the 3′ terminus, segregating with cortical myoclonus in eight affected individuals. This configuration shows meiotic stability but low-level somatic variability in blood. We observed an inverse correlation between the number of TTTCA repeats and the age at myoclonus onset.

Conclusion: These findings indicate that as little as five TTTCA repeats combined with expanded TTTTA repeats can cause cortical myoclonus without epilepsy, highlighting potential mechanisms underlying FAME pathophysiology.

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P11.016.C Positive and negative affect, related mental health traits, and cognitive performance: shared genetic architecture and potential causality

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Background: Altered affect and cognitive dysfunction are features of many neuropsychiatric conditions which are highly comorbid, remain poorly understood, and have few efficacious treatments. Exploring their genetic architecture and causal relationships may provide insights into aetiology and comorbidity.

Material and Methods: Using Lifelines Cohort data, we conducted the first genome-wide association studies (GWAS) on positive and negative affect using a validated measure ($N = 57{,}946$) and four cognitive domains (working memory, reaction time, learning and memory, executive function; $N \geq 35{,}729$). We used genetic correlation and bidirectional Mendelian randomization (MR) to assess genetic overlap and causality, incorporating large GWAS on related - albeit distinct - phenotypes (depression, anxiety, wellbeing, general cognitive ability [GCA]).

Results: We identified one GWAS hit for reaction time, and many loci with suggestive associations for other phenotypes ($N = 11$–20), including positive ($N = 20$) and negative ($N = 15$) affect. For most phenotypes, suggestive hits have higher gene expression in brain tissue compared to other tissues. Genetic correlations between negative and positive affect suggest they are dissociable constructs ($r_g = -0.18$). GCA has higher genetic

overlap with negative affect ($r_g = -0.19$ versus -0.06) which could indicate that negative affect and GCA have a higher shared neural basis and/or they exhibit causal relationships. Supporting the latter, MR analyses suggest higher GCA may reduce negative affect, depression and anxiety, and increase wellbeing, with little impact on positive affect. Conversely, depression and lower wellbeing may causally reduce GCA.

Conclusion: These results suggest GCA may be a valid target to prevent negative affect; and depression/wellbeing may be valid targets for GCA.

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P11.017.D Cell-type and developmental specificities of rare variants underlying psychiatric co-occurrences in autism

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Background: Co-occurring psychiatric diagnoses are frequent in autism spectrum disorder (ASD) and significantly impact quality of life. Whether their genetic contributors and brain processes overlap or are distinct from those of autism remains unknown. To investigate this, we developed a cell-type[1] burden approach that enhances power to test associations between rare inherited variants and traits while providing a functional readout.

Material and Methods: Using exomes from 43,531 autistic individuals from the SPARK cohort[2], we analyzed rare (MAF <0.001), loss-of-function and missense variants in intolerant genes (LOEUF < 0.6). Associations with 11 psychiatric diagnoses were tested by functional burden ; aggregating variants across cell-types and developmental epochs. Psychiatric diagnoses included: ADHD (n = 14,342); anxiety (n = 9,013); sleep disorders (n = 7,295); depression (n = 4,769); obsessive-compulsive (n = 3,884); disruptive behavior disorder (DBD) (n = 3,539, including oppositional defiant (ODD, n = 2,872)); bipolar (n = 1,284); tics (n = 1,205); schizophrenia (n = 487) and eating disorders (n = 371).

Results: ADHD, ODD and DBD exhibited a significant missense variants burden, particularly in inhibitory interneuron subtypes. For ADHD, ODD and DBD, respectively 33%, 81% and 60% of the missense variants signals were distinct from autism liability. ODD also showed loss-of-function variant signals in inhibitory interneurons. No significant burden was observed for other diagnoses, which may be partially attributed to limited statistical power.

Conclusion: These findings highlight the cell-specific impact of rare SNVs on psychiatric co-occurrences in ASD, with interneurons playing a central role. This enhances our understanding of the genetic and cellular underpinnings of psychiatric diagnosis in autism and reveals that part of this liability is distinct from autism susceptibility.

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P11.018.A Identification and rescue of circadian rhythm defects in Prader-Willi syndrome neuronal cultures

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Background: Prader-Willi Syndrome (PWS) is a multigenic neurodevelopmental disorder characterized by loss of expression of maternally imprinted genes in the 15q11.2-q13.1 region. PWS individuals present with hypotonia, developmental delay, childhood obesity, and sleep disorders. Little is known about the molecular mechanism during development which leads to excessive daytime sleepiness in PWS. Since current mouse models do not accurately recapitulate PWS phenotypes, cellular models from PWS patient samples are crucial to understanding the molecular etiology of disease during neural development.

Materials: We use dental pulp stem cell (DPSC) derived neuronal cultures to investigate a variety of neurodevelopmental disorders including PWS. These neuronal cultures contain a collection of cell types including glia, excitatory and inhibitory neurons.

Results: We have shown that *Per2*:luc DPSC-derived neuronal lines from PWS individuals have a shortened Per2 cycling period lengths. This shorter period was rescued to neurotypical control using the CKI inhibitor called longdaysin. Alongside these patient cell lines, we are employing *CRY1*-fluorphore-tagged U2OS lines to study MAGEL2's role in circadian cycling through CRY1 regulation. We will treat these cells using different pharmacological agents to see how modulating components downstream from MAGEL2 affects cycling in live cells.

Conclusions: These studies provide insights on how loss of normal MAGEL2 regulation of circadian cycling in PWS leads to excessive daytime sleepiness. Understanding how components downstream from MAGEL2 are modulated pharmacologically will provide new knowledge for future PWS sleep-related treatments of both PWS and the related disorder Schaaf-Yang Syndrome.

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P11.019.B Non-canonical BRD4 function rescues the molecular disease signature of X-linked dystonia parkinsonism

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Background: X-linked Dystonia-Parkinsonism (XDP) is a rare, neurodegenerative disorder, which predominantly affects males with a Filipino ancestry. The disease-associated phenotype results from the insertion of an SVA retrotransposon within intron 32 of the *TAF1* gene, encoding the largest subunit of the basal transcription factor TFIID. This insertion determines aberrant processing of *TAF1* mRNA synthesis, including intron 32 retention and incorporation of intron 32 sequences into mature *TAF1* mRNAs, together with a reduction in full-length mRNAs.

Material and Methods: To understand the molecular mechanism of XDP-associated transcriptional dysregulation, we developed a fluorescence- and cell-based reporter assay, which recapitulates the XDP molecular signature, and employed this assay as a screening platform for chemical libraries focused on epigenetic pathways. To validate the identified hits, we applied patients-derived neural progenitor cells and established XDP cerebral organoids.

Results: The screening led to the identification of BRD4 as a critical regulator of XDP-SVA-dependent defects in *TAF1* mRNA processing. Interestingly, the acetyl-lysine binding function of BRD4 is not essential for rescue of the XDP molecular signature, which requires instead non-canonical BRD4 functions associated with mRNA processing and termination. Similarly, defective *TAF1* mRNA processing in XDP patient-derived cell lines and cerebral organoids is also countered by BRD4 depletion.

Conclusion: Here, we use an unbiased, comprehensive screening approach to uncover a novel disease mechanism that links XDP pathogenesis to defecting transcription termination, which uncovers a non-canonical function of the transcription regulator BRD4.

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P11.020.C Involvement of *CACNA2D2* in developmental and epileptic encephalopathy through disruption of calcium channel functionality and synaptic function

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Background: Developmental and epileptic encephalopathy (DEE) associated with *CACNA2D2* variants is an ultra-rare autosomal recessive disorder with a strikingly homogeneous phenotype. *CACNA2D2*, predominantly expressed in the cerebellum, encodes the $\alpha_2\delta$-2 subunit of voltage-gated calcium channels (VGCC). It regulates channel membrane trafficking and current properties. We aim to expand the clinical description and to assess the functional impact of relevant *CACNA2D2* variants.

Methods: Through GeneMatcher, ERN-EPiCARE and international collaborations, we assembled clinical and genetic information of patients carrying biallelic *CACNA2D2* variants. Functional characterization, including minigene assays and protein expression analysis of relevant novel variants were performed.

Results: We report clinical and genetic data of a new larger case series of 22 new individuals harboring *CACNA2D2* variants along with 9 previously reported cases. The cardinal manifestations included early-onset drug-resistant epilepsy, severe cognitive and motor impairment, and cerebellar atrophy. Facial and limb dystonia was observed in two families, while ataxia was absent in the newly reported patients. Molecular analysis identified 17 *CACNA2D2* variants, comprising 7 missense and 10 truncating variants (6 splicing, 3 nonsense, and 1 frameshift deletion). Functional studies demonstrated distinct pathophysiological mechanisms: missense variants affect the functional expression of VGCC, alter synaptic functions while truncating variants are likely triggering nonsense-mediated RNA decay, and lead to a complete loss of protein. Our data predict that aberrant expression of α2δ-2 disrupts the excitatory-inhibitory synaptic balance, a key mechanism underlying neurodevelopmental disorders.

Conclusion: Our findings strengthen the role of *CACNA2D2* in DEE and highlight the dual pathogenic mechanisms underlying this disorder, encompassing both channelopathy and synaptopathy.

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P11.022.A Enhancing Parkinson's Disease Risk Prediction: Combining Polygenic Risk Scores with Transcranial Sonography

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Background: Parkinson's Disease (PD) affects approximately 3% of individuals aged 65 and older. Currently, PD is diagnosed only when symptoms manifest. The polygenic risk score (PRS) can identify individuals with a very high genetic risk for PD, but its lack of specificity limits its clinical usefulness. Therefore, additional tests are needed for individuals with extremely high PRS. In this study, we combine PRS with transcranial sonography (TCS), utilizing signals from the substantia nigra (SN) to enhance PD risk prediction and potentially justify earlier intervention.

Material and Methods: PD-PRSs were calculated for over 41,000 participants aged 55 years and older from the Estonian Biobank, all without a prior PD diagnosis. Participants in the highest and lowest PD-PRS percentiles (n = 222) underwent TCS measurements. After identifying the butterfly-shaped mesencephalic brainstem, we located the maximal ipsilateral SN area in the axial plane. Images were captured, manually labeled, and planimetry was conducted.

Results: Out of 222 participants in the high-risk group, 9 (8.4%) were diagnosed with PD during the initial visit. In contrast, 3 (3.1%) individuals in the low PD-PRS group received new PD diagnoses. The most significant difference between the high- and low-risk groups was observed in SN hyperechogenicity (SN+) area, with mean values of 0.21 cm^2 for the high-risk group and 0.15 cm^2 for the low-risk group ($p < 0.001$).

Conclusion: Our multivariate analysis indicated that individuals with the highest PD-PRS had nearly six times higher odds of exhibiting SN+ compared to those with the lowest PD-PRS.

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P11.023.B The functional minisatellite MNS16A at *TERT* locus associates with Alzheimer's disease risk and related endophenotypes

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Background: Polymorphic DNA minisatellites have been largely overlooked in genome-wide association studies (GWAS), despite their potential significance in shaping genetic traits and contributing to disease susceptibility. Among these, MNS16A is a functional minisatellite located within the *TERT* locus, which previous studies have linked to longevity, Alzheimer's disease (AD), and cancer. The objective of our study was to examine the association between MNS16A, AD, and its related endophenotypes.

Material and Methods: MNS16A was genotyped by PCR in two independent Italian cohorts of European ancestry, comprising 828 individuals (434 cases and 394 controls). The association between MNS16A and AD risk was assessed using logistic regression, adjusting for *APOE* genotype, sex, and age, with results fixed-effects meta-analysed across the cohorts. Additionally, the relationship between MNS16A and AD-related endophenotypes, including leukocyte telomeric length (LTL), Tau-cerebrospinal fluid (CSF), and Tau-PET, was investigated in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort using linear mixed models.

Results: MNS16A*S allele is associated with a reduced risk of AD (OR = 0.793, P = 0.029, I^2 =0.00%), longer LTL (beta = 0.030, P = 0.032) and reduced levels of Tau in both CSF (beta = −0.066, P = 0.032) and PET (Braak 5-6 SUVR, beta = −0.028, P = 0.049)

Conclusion: Our findings confirmed the association of the MNS16A*S allele with a reduced risk of AD. Furthermore, we were the first to associate the MNS16A*S allele with LTL and Tau levels in both CSF and PET imaging, reinforcing the protective role of the *S allele in AD-related endophenotypes. Further research with larger cohorts is needed to validate and extend these findings.

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P11.024.C A Comprehensive Genotype-first Analysis of Potassium Channel Variants: Insights into Kv Channelopathies in a Multi-ethnic Cohort of 40,000 Exomes

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Background: Potassium ion channels, particularly voltage-gated (Kv) channels, are essential for repolarizing cell membrane potentials and propagating electrical signals. Defects in Kv channels, known as Kv channelopathies, are associated with a broad spectrum of neurological and cardiac disorders. Despite their clinical significance, these conditions remain poorly understood, especially in underrepresented populations.

Materials and Methods: We analysed 15 Kv genes with established neurological gene-phenotype associations using a multi-ethnic database of 40,000 exomes, including patients with neurological disorders and controls. Variants were filtered and classified using ACMG guidelines into Benign, Likely Benign, low/medium/high Variants-of-Uncertain-Significance (VUS), and Likely Pathogenic to Pathogenic (LP/P) categories. Gene constraint metrics were applied to assess gene variation intolerance and normalize variant loads across genes by size. Patients with high VUS or LP/P variants underwent phenotype correlation, full exome re-analysis, and segregation testing via Sanger sequencing or trio WES, when available.

Results: A total of 1,609 variants were classified across 15 Kv genes, with significant clustering of high VUS to LP/P variants in genes and regions with high constraint scores, particularly in voltage-sensing and intramembrane helices. LP/P variants were identified in controls or individuals with irrelevant phenotypes, suggesting incomplete penetrance. Out of 25 variants selected for segregation analysis, 15 were confirmed pathogenic, including one indicating a novel mode of autosomal recessive inheritance.

Conclusion: This genotype-first study of Kv channelopathies integrates gene constraint metrics and segregation data, advancing our understanding of variant pathogenicity and genotype-phenotype correlations. These findings provide crucial insights into Kv channelopathies, particularly in diverse and underrepresented populations.

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R. Lee: None. **R. Maroofian:** None.

P11.025.D SLC6A4 genotype predicts telomere shortening in depression more persistently than psychological factors

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Background: Environmental and psychological stress accelerates telomere shortening. Psychiatric disorders, especially those triggered by stress, can alter telomere length. Genetic factors associated with depression vary across cultures and environments. The serotonin transporter gene *SLC6A4* VNTR polymorphism affects susceptibility to psychiatric disorders differently in different regional populations. This study aimed to investigate the correlation between telomere length and depression in Indonesia, focusing on socioeconomic, cultural, and genetic factors amidst rapid social changes.

Material and Methods: We recruited 99 psychiatric outpatients and 101 participants without depressive symptoms of 16 to 76 years of age. Psychological assessments were conducted using the Self-Construal Scale (SCS) and Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) via interviews. DNA extracted from the peripheral blood was used to identify the *SLC6A4* genotype by PCR, and telomere length was measured by quantitative PCR.

Results: The comparison of patients with depression and controls revealed significant telomere shortening in the patient group ($p < 0.0001$, Mann-Whitney U-test). Logistic regression showed that psychological scales were stronger predictors of depression than telomere length or *SLC6A4* polymorphisms ($p < 0.01$). A multiple regression analysis indicated that the LL genotype of *SLC6A4*, but not socioeconomic or psychological factors, was strongly associated with telomere length ($p < 0.005$).

Conclusion: Psychological factors were stronger predictors of depression than telomere length or SLC6A4 polymorphisms. However, the LL genotype of SLC6A4 was significantly associated with telomere length, independent of socioeconomic or psychological factors.

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P11.026.A Comprehensive genetic analysis of an Italian Amyotrophic Lateral Sclerosis cohort

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Background: Amyotrophic Lateral Sclerosis (ALS) is the most prevalent motor neuron disease in adults, affecting approximately 1 in 350 individuals. It is caused by progressive degeneration of upper and lower motor neurons, leading to muscle atrophy, weakness, stiffness, and progressive paralysis, resulting in respiratory failure within 3–5 years of onset. ALS has a complex genetic architecture, involving monogenic, oligogenic, and polygenic inheritance. About 10% of ALS cases have a family history, with pathogenic mutations identified in over half of these cases. Sporadic ALS accounts for the remaining cases, with heritability estimated at 40–50%. Over 23 genes have been linked to ALS, with *C9orf72*, *SOD1*, *FUS*, and *TARDBP* collectively responsible for 60–70% of familiar cases.

Material and Methods: A combination of repeat-sizing of C9orf72 and whole-exome sequencing (in silico panel including genes) was used to comprehensively assess genetic variations in 439 Italian patients with ALS. Clinical and genetic data have been compared to assess the genetic contribution to the pathogenesis of familial and sporadic ALS.

Results: Pathogenic, likely pathogenic, and uncertain significance variants were identified in 15 genes, with *C9orf72* (7%), *SOD1* (3%), *FUS* (2%), and *TARDBP* (2%) being the most common. Overall, 23.7% of patients had a detectable genetic variant. Phenotype-genotype correlation revealed that patients with *C9orf72* expansions had poorer survival compared to those with other mutations or no mutations in the ALS panel genes.

Conclusion: These findings align with other high-throughput studies on the Italian ALS population, highlighting the genetic complexity of ALS and its role in clinical outcomes.

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P11.027.B Genomic analysis of the overlap between internalizing and externalizing behaviors

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Background: Internalizing problems, such as anxiety and depression, and externalizing behaviors, such as aggression and rule-breaking, often emerge early in life and are associated with adverse adult outcomes. A notable comorbidity exists between externalizing and internalizing psychopathology. Understanding the shared genetic factors is essential for comprehending their biological mechanisms.

Material and Methods: This study investigates the polygenic overlap between internalizing and externalizing behaviors using large-scale genome-wide association studies (GWAS) of internalizing behaviors (N = 64,641 children/adolescents) and externalizing behaviors (N = 1.5 million adults). We employed linkage disequilibrium score regression (LDSC), to explore SNP-heritability, genetic correlations, and FUMA to explore gene expression based on single nucleotide variants (SNVs).

Results: LDSC analyses revealed that a small proportion of SNVs explain these phenotypes (SNP-heritability: internalizing = 1.7%; externalizing = 6%). The genetic correlation between these traits was not significant (rg = 0.065, p = 0.27). FUMA identified 757 genes expressed in externalizing behaviors and 29 with internalizing behaviors, being that expression of these genes is downregulated (considering differentially expressed genes in both sides) and were enriched for cerebellar tissue and the cerebellum (GTEx data). Eight overlapping genes (e.g., *ARL17B, CRHR1, KANSL1, LRRC37A, MAPT, PLEKHM1, SPPL2C, STH)* were involved in neural processes, including the tubulin complex pathway (GO:0045298, adjusted-p-value = 0.049).

Conclusion: Our preliminary findings suggest that internalizing and externalizing phenotypes are partially explained by SNVs, although these SNVs are not genetically correlated. Shared genes may underlie these behaviors, warranting further causal studies to clarify their genetic relationship.

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P11.028.C Further evidence of biallelic variants in *HACE1* as a cause of Spastic Paraplegia and Psychomotor Retardation

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HACE1 encodes a HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase, regulating several small GTPases. This protein is involved in several important functions, such as cell division, protein ubiquitination, and localization. Biallelic defects in *HACE1* have been implicated in spastic paraplegia and psychomotor retardation with or without seizures (OMIM 616756). Previously, 24 patients of various ethnicities have been reported with a range of variant types; pathogenic, likely-pathogenic, and variants of uncertain significance. Here, we studied five unrelated families of diverse ethnicities with eight patients of 10 months-to-30 years of age. Our patients presented global developmental delay, motor delay, dysarthria, dysphagia, and late-onset seizures. Exome Sequencing identified biallelic variants in the gene *HACE1*. Four out of the five families harbour biallelic loss-of-function variants (Family 1: *HACE1* (NM_020771.4): c.1396C>T p.(Gln466*); Family 2: c.2628-1G>C p.(?); Family 3: c.355G>T p.(Glu119*)); Family 4 carries a compound heterozygous nonsense variants (c.2242C>T p.(Arg748*)/c.152C>G p.(Ser51*)) while a missense variant (c.2711A>G p.(Tyr904Cys)) was identified in the Family 5. All variants segregated with the phenotype in respective families and are classified as pathogenic/likely pathogenic by ACMG criteria except the missense variant p.(Tyr904Cys) which is a VUS. This study strengthens and expands the genotype spectrum of *HACE1*-related rare neurodevelopmental disorder. The addition of our cohort will be helpful in understanding the genotype-to-phenotype correlation. Further, we provided genetic diagnosis to the participating families which will aid in genetic counseling and parental decision-making in the future.

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P11.029.D *Up-regulation of a tRNA-derived small non coding RNA in tears of Parkinson's disease patients*

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Background: Parkinson's disease (PD) is a neurodegenerative disorder with motor and non-motor symptoms. Diagnosis remains challenging, requiring post-mortem confirmation. Tear fluid (TF) reflects ocular and central nervous system states, making it a promising non-invasive biomarker source. This study analysed TF miRNA profiles in PD patients compared to healthy controls (HC) to assess their potential for improving early diagnosis and disease monitoring.

Material and Methods: The study included a discovery (8 PD, 7 HC) and a validation cohort (9 PD, 9 HC). We assessed miRNA expression using TaqMan Human MicroRNA arrays, and their associations with clinical variables. Due to small sample size, a fold change threshold (95th percentile in HC) was set, classifying patients as above or below threshold. Composite scores of clinical variables were compared. Target gene prediction, pathway enrichment, and functional interaction analyses were conducted.

Results: We identified a miRNA upregulated in PD tears across two cohorts, reported as tRNA-derived with gene-regulation function. Its expression was higher in a PD subgroup with combined non-motor autonomic symptoms. Target genes are linked to neurodegeneration and cellular dysfunction. Over-representation analysis highlighted its role in the negative regulation of synaptic transmission, while network analysis revealed interactions among key PD-related genes and miRNA targets, suggesting its involvement in disease mechanisms.

Conclusion: Our findings suggest that TF miRNA profiling can identify PD-specific molecular signatures, highlighting its potential as a non-invasive source for early diagnosis. Further studies with larger cohorts and other neurodegenerative diseases are needed to validate these results and explore their clinical applicability.

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P11.030.A Characterization of an SPG9 cell model to investigate hereditary spastic paraplegia: cell phenotyping and potential treatment

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Background: Spastic Paraplegia type 9 (SPG9) is a rare genetic neurodegenerative disorder caused by mutations in the ALDH18A1 gene. In this study we've derived a cellular model based on a patient-derived fibroblast cell line carrying an

ALDH18A1 pathogenic variant (NM_002860.4:c.727G>C, p.Val243-Leu). Our investigation focuses on elucidating fundamental cellular properties, including cell dimensions, growth dynamics, and mitochondrial features, with the aim of gaining insights into the pathological mechanisms underlying SPG9. Furthermore, our in vitro model offers a unique scorable phenotype that can be used for rescues experiments and to identify novel therapies.

Material and Methods: Initial analyses of the SPG9 cell model revealed distinct phenotypes that are the results of alterations in cell dimensions, growth pattern, and mitochondrial features such as ROS production, ATP synthesis, by phalloidin staining, MTT analysis, galactose growth compared to WT cells. Furthermore, we explored a therapeutic approach by investigating the effects of amino acid supplementation on our in vitro model.

Results: Preliminary results suggest that it is possible to identify specific cell phenotypes, and that specific amino acid supplementation may hold promise as a potential treatment strategy for SPG9, offering a targeted approach to mitigate cellular dysfunction associated with *ALDH18A1* gene mutations.

Conclusion: Our study provides a comprehensive understanding of the cellular characteristics of SPG9, emphasizing the potential utility of amino acid supplementation as a therapeutic intervention. Moreover, our findings contribute to unravel the molecular mechanisms underlying SPG9 and pave the way for the development of targeted therapies for this rare debilitating neurodegenerative disorder.

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P11.031.B The missing heritability of SPG7: a part of the answer in the detection of copy number variant

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Background: Spastic paraplegia 7 (SPG7) represents the most frequent autosomal recessive spastic paraplegia. The phenotype picture is either pure or complex encompassing ataxia, optic atrophy, and parkinsonian features. Dominant inheritance has been reported in the literature, associated with specific variants, but it remains a subject of debate.

Material and Methods: We analysed genomic data from 74 patients from SPATAX/BIOMOV cohorts at the Paris Brain Institute (ICM) with a monoallelic variants in SPG7 and a phenotype compatible with SPG7. Copy number variant (CNV) analyses were performed on exome data using the Illumina Dragen software CNV pipeline.

Results: We identified seven patients (9.4%) carrying a deletion and the Ala510Val variant. A recessive transmission was described for two cases, the other five were sporadic. The age at onset was 43 ± 6.3 years, with unsteadiness and/or stiffness as the first signs. The pure form of SPG7 accounted for 30% and complex form for 70% associating with neuropathy, cerebellar ataxia, and oculomotor signs. No patients exhibited cognitive impairment or parkinsonism. MRI revealed cerebellar atrophy in four patients.

Conclusion: The detection of a CNV enabled the diagnosis of SPG7 in 9.4% of our cohort of heterozygous SPG7 carriers. The apparent heterozygous state of affected patients could be due to an insufficient variant coverage in SPG7 with previous techniques (e.g., NGS panel or WES), which hides a second pathogenic variant such as a CNV, an intronic variant, etc. Additionally, another explanation could be the presence of a second causative gene, specifically impacting the mitochondrial respiratory complex.

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P11.032.C Genetic mosaicism in diffuse Malformations of Cortical Development: Insights and Implications in the diagnostic workflow

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Background: Malformations of cortical development (MCDs), whether autosomal or X-linked-dominant, are often caused by de novo pathogenic variants, with somatic mosaicism also playing a contributory role. We aimed to assess the prevalence of somatic mosaicism in MCDs and to optimize the diagnostic protocol to identify mosaic pathogenic variants in both probands and parents of MCD patients carrying alleged de novo variants.

Material and Methods: We performed deep amplicon sequencing (DAS) with a read depth above >2,500 (mean depth: 87,071) on blood-derived DNA from 93 patient-parent trios (279 individuals) harbouring presumed de novo pathogenic variants in 31 MCD-associated genes, previously identified by targeted gene panel or exome sequencing. Amplicon libraries were prepared and sequenced using a 2×150 bp paired-end protocol on Illumina platforms. To detect mosaic variants, we developed MosDetection, a customized script that reprocessed bam-read-count output with adjustable parameters for SNVs and indels, providing sequencing depth and alternative/reference read counts for each trio.

Results: Mosaicism was identified in six unrelated individuals: four probands with variant allele frequencies (VAFs) ranging from 18% to 35% and two parents with VAFs of 3% and 8%, respectively. Notably, parents carrying these somatic pathogenic variants were asymptomatic.

Conclusion: Somatic mosaicism was identified in 6.4% of the cohort (6/93 trios), including 4.3% in probands (4/93 trios) and 2.1% in parents (2/93 trios). Our findings demonstrate that DAS combined with the MosDetection tool effectively detects low allele frequency variants. This approach enhances segregation analysis, mosaic variant identification, and VAF quantification, thus improving genetic counseling and diagnostic workflow.

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P11.033.D Genetic and transcriptomic insights into major depressive disorder heterogeneity

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Background: Major depressive disorder (MDD) affects 5% of the population, impairing psychosocial functioning and quality of life. Its phenotypic heterogeneity complicates identifying genetic determinants, requiring advanced patient stratification and interpretable methods.

Material and Methods: Phenotypic stratification was conducted using a consensus clustering approach on MDD individuals from the UK Biobank (UKB). Two stratification models were applied: (i) multimodal neuroimaging (n = 7948); (ii) clinical information on depressive symptomatology (n = 35033). A Transcriptome-Wide Association Study (TWAS) was performed using PrediXcan. Clusters were characterized through Mann-Whitney-U and chi-squared tests for clinical variables, and logistic regression models for genes and pathways. RNA sequencing (RNAseq) was conducted using DESEQ2 on an independent MDD cohort (n = 83), stratified with a stability-based clustering on structural neuroimaging.

Results: Two distinct clusters were identified in both UKB datasets, differing in cardiovascular comorbidities, diabetes, and depression severity. Clinical clusters varied in treatment resistance and the predicted expression of NDUFAF3, GPX1, and DPPA4, while neuroimaging clusters differed in PABPC1 and FYN. Enriched pathways indicated roles in chemical stress response and chromatin remodeling. Two clusters were also found in the independent cohort, with RNAseq identifying 39 differentially expressed genes, including SOCS3, a trait marker for depressive symptoms, and MMP8, associated with stress.

Conclusion: This study demonstrates the existence of two homogeneous MDD groups linked with specific gene expression patterns, found in independent samples and using different strategies, providing insights on the molecular mechanisms for tailored therapeutic approaches. This research used the UKB (Application 56514) and was supported by the Italian Ministry of Health (GR-19 0616).

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P11.034.A Investigating TANGO2's role in mitochondria and neuronal activity using patient-derived iGluNeurons

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Background: Biallelic *TANGO2* loss-of-function variants cause a multisystemic disorder with metabolic-encephalomyopathic crises, rhabdomyolysis, and neurodegeneration. We generated induced-Pluripotent-Stem-Cells(iPSCs) from two siblings with the same genotype (c.262C>T(p.Arg88*);c.338delG(p.Gly113Alafs*10)) but exhibiting different clinical symptoms. Symptomatic patient-derived neurons showed increased oxidative stress, whereas asymptomatic sibling's cells remained comparable to controls. To further investigate, we examined TANGO2's role in mitochondrial redox-imbalance and synaptic connectivity in induced-glutamatergic-Neurons(iGluNeurons).

Material and Methods: iPSCs from TANGO2-patients and controls were differentiated into iGluNeurons via neurogenin-2-inducible overexpression. Mitochondrial membrane potential and elongation were evaluated with tetramethylrhodamine-ethyl-ester(TMRE) dye, while electrophysiological activity was recorded using High-Density Multi-Electrode-Array(HD-MEA). Neurotransmission changes were performed with glutamate assay.

Results: At 22 days in-vitro(DIV), no significant differences in mitochondrial membrane potential were observed, likely because this early maturation stage is too premature to reveal TANGO2-deficiency effects. Further analyses will exhibit energy maintenance at later stages. However, mitochondrial length increased in both patient-iGluNeurons, with slight elongation in the asymptomatic ones, highlighting enhanced fusion processes, potentially as a cellular stress response. Electrophysiological analysis (30DIV) showed that symptomatic patient-iGluNeurons exhibited a significant reduction in firing rate, burst rate, and duration compared to controls, suggesting impaired neuronal activity, and signaling. Additionally, glutamate release was reduced at 21DIV, further confirming synaptic dysfunction. Asymptomatic patient analysis is ongoing.

Conclusion: Increased mitochondrial fusion suggests an initial compensatory mechanism, more effectively sustained in the asymptomatic patient. Subsequent studies will explore whether asymptomatic patient's compensation involves enhanced ATP production and mitophagy. Electrophysiological impairment and reduced glutamate release observed underscore TANGO2's role in synaptic transmission and neuronal signaling.

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P11.035.B No association between *FMR1* premutation and either ADHD or anxiety in 53,707 women undergoing genetic testing for family planning purposes

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Background: *FMR1* premutation has been inconsistently associated with neuropsychiatric phenotypes, possibly due to

ascertainment bias. We investigated the association between *FMR1* premutation and attention deficit hyperactivity disorder (ADHD), anxiety, and other psychiatric disorders in large-scale population-based genetic carrier screening data.

Material and Methods: We defined premutation as having 58–200 CGG repeats. Phenotypes were identified in linked electronic medical records via formal diagnoses or relevant medication purchases. As a positive control, we assessed premature ovarian insufficiency (POI) and elevated follicle-stimulating hormone (FSH) levels before the age of 40.

Results: Our study included 53,707 women, among them 464 premutation heterozygotes. The premutation status was associated with POI (hazard ratio [HR]: 4.08, 95% confidence interval [CI]: 2.16–7.72) and high FSH (HR: 3.43, 95% CI: 2.65–4.43) but not with ADHD (HR: 1.08; 95% CI: 0.75–1.56), anxiety (HR: 0.74; 95% CI: 0.53–1.04), anxiety and depression (HR: 0.86; 95% CI: 0.69–1.07), and other psychiatric disorders (HR: 1.22; 95% CI: 0.73–2.03). Our study was sufficiently powered to detect HR in the range approximately 1.5–2.

Conclusion: No association was found between FMR1 premutation status and either ADHD or anxiety. While our study design avoided bias towards affected families, participants may be healthier than average, and small effects cannot be excluded.

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P11.037.D Importance of stereotypical migration routes for precise cortical interneuron positioning and circuit formation

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Background: Cortical GABAergic interneurons (cIN) are crucial for the proper function of cortical circuits. Incorrect numbers and defects in synaptic integration are linked to disorders such as epilepsy, schizophrenia, and anxiety. These interneurons originate in the ventral telencephalon and migrate into the developing cortex in two streams that are hypothesized to be key for their proper positioning and subsequent integration into the developing cortex.

Material and Methods: We utilized a Cxcr4 conditional knockout (cKO) mouse model to investigate the impact of disrupted stereotypic migration on cortical development. We analyzed cIN distribution, gene expression by single cell RNA-seq, and synaptic connectivity throughout embryonic and postnatal development.

Results: Despite reaching most cortical regions, Cxcr4-deficiency disrupts cIN migration within the sub-meningeal compartment, resulting in a permanent deficit of cINs in superficial layers. Aberrant positioning shifts cIN identity towards a somatostatin subtype and reduces expression of synaptic genes. Furthermore, GABAergic synapses preferentially target deep layers, mirroring altered cIN distribution.

Conclusion: Cxcr4-regulation of layer-specific cIN migration is essential for proper cIN allocation, maturation, and ultimately, formation of cortical circuits. Disruptions in this process may contribute to the pathophysiology of neurological and mental disorders.

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P11.038.A A single-cell eQTL atlas of the human cerebellum reveals vulnerability of oligodendrocytes in essential tremor

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Background: Essential Tremor (ET) is the most common movement disorder, affecting approximately 1% of the worldwide population. It is characterized by an active, postural, or kinetic tremor, primarily affecting the upper limbs, which can be very severely debilitating to quality of life. The pathophysiological mechanisms of ET are largely unknown, and despite strong evidence of heritability, studies of ET genetics have yielded mixed results.

Material and Methods: We sought to understand the effects of genetic risk factors in ET on the cerebellum by building a population-scale single-cell atlas of the human cerebellar cortex comprised of over 1 million cells from 109 individuals. We utilized single-cell expression quantitative trait loci (eQTLs) and Mendelian randomization (MR).

Results: We found evidence of downregulation of BACE2 in cerebellar oligodendrocytes caused by ET-associated variants in its locus. We also highlight a genetically vulnerable population of BACE2-expressing immature oligodendrocytes, which suggests demyelination. Additionally, we find dysfunctional interactions between Golgi cells, Purkinje layer interneurons, and oligodendrocytes in ET.

Conclusion: Our study suggests a putative disease model where oligodendrocytes play a crucial role in the pathogenesis of ET.

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P11.039.B Integration of long-read nanopore sequencing for precision resolution of genomic variants in dystonia

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Background: Dystonia is a movement disorder characterized by monogenic aetiology in a relevant proportion of cases, with more than 400 disease-associated genes described to date. Because of well-known limitations regarding allele phasing, structural variant detection, short-tandem repeat analysis, and scrutiny of high-homology sequences, short-read sequencing (SRS) technologies are incapable of comprehensively characterising the full spectrum of genome variation. It is therefore crucial to investigate the feasibility of alternative diagnostic approaches, such as long-read DNA sequencing (LRS), which promise to overcome several unsolved challenges of SRS.

Material and Methods: We selected seven unrelated dystonia patients in which candidate variants could not be conclusively interpreted by SRS methods. LRS was performed on patients' native DNA fragments using an Oxford Nanopore Technology (ONT) platform according to the manufacturer's protocols. Bioinformatic analysis was performed using a pipeline supporting the calling of single-nucleotide variants, structural variants, short-tandem repeats, and modified bases.

Results: LRS analysis offered precise molecular characterization of each patient, resolving previous indeterminate findings. In two cases, phasing analysis proved compound heterozygosity of pathogenic variants (*HIBCH*, *OTUD7A*). In two other patients, previously unresolved complex structural variants were thoroughly characterized (*MECP2*, *SCGE)*. Accurate estimation of repeat-expansion length was critical for diagnosis in two additional subjects (*TBP*, *FMR1*). Finally, we re-evaluated one case with a suspicious variant call in a gene for which a pseudogene with high sequence similarity exists (*PI4KA*).

Conclusion: ONT-based LRS represents a promising tool for complementing SRS and resolving inconclusive results in the diagnosis of genetic forms of dystonia.

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P11.040.C Memory disorders in Neurofibromatosis type I

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Background: Neurofibromatosis type I (NF1) is a neurocutaneous syndrome commonly associated with cognitive deficits, including memory disorders and learning disabilities. Among the neurobiological mechanisms involved are alterations in long-term potentiation (LTP) and volumetric changes in the hippocampus, a structure crucial for both long-term and short-term memory processes.

Material and Methods: The retrospective study involved 111 patients with NF1, mean age 22.76 years (range 5–63 years). A subgroup of 7 patients aged 5–16 years along with 10 healthy controls, underwent neuropsychological testing using the NEPSY battery (Developmental Neuropsychological Assessment), a standardised instrument that assesses specific aspects of verbal and non-verbal memory. In parallel, T1-weighted three-dimensional MRI images were used to calculate hippocampal volumes using validated neuroimaging software.

Results: 71% of the patients demonstrated difficulties with verbal and non-verbal memory tasks. Specifically, increased left hippocampal volume was associated with poorer performance on certain memory tests ($p < 0.05$). This correlation highlights a direct link between structural alterations and cognitive deficits in NF1 patients.

Conclusion: The results suggest that volumetric changes in the hippocampus negatively affect memory abilities in NF1 patients, with clear implications for the neurocognitive profile of this population. The use of the NEPSY battery provided a deeper understanding of the specific difficulties, underscoring the need for further studies aimed at developing interventions to improve patients' quality of life.

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P11.042.A Exome sequencing in the diagnosis of pediatric neurogenetic disorders: An Indian perspective

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Background: Neurogenetic disorders are a large group of heterogeneous conditions with wide variability in age of onset, clinical presentation, and severity. Diagnosing these disorders is challenging due to a multitude of factors. Exome and genome sequencing have helped us overcome some of these to make a diagnosis.

Material and Methods: This is an ambispective study conducted over five years at a tertiary care center in India. We included patients with suspected monogenic disorders and recruited 3,914 families after excluding non-Mendelian disorders, subjecting them to exome sequencing. 3,856 underwent proband-only, while the rest opted for a trio exome.

Results: Males constituted 55.9%. Consanguinity was noted in 31.5% (1,232 cases). Positive family history of similarly affected siblings was seen in 5.6% (216 families). The overall diagnostic yield comprising matching pathogenic/likely pathogenic variants was 47.7% (1,867 cases). Novel mutations were seen in 54.6%. Multiple genetic diagnoses were obtained in 256 (6.54%): dual diagnosis was more commonly seen in 230 families, triple diagnosis in 22, four disorders in 2, and five disorders in 2 each, respectively. Autosomal dominant and recessive disorders comprised the majority; founder mutations were found in 15 genes, and one novel gene, *LARP1B*, was found to cause neurodevelopmental disorder phenotype. A novel phenotype in 12 genes was

noted. Expansion of phenotype was reported across 88 OMIM-related diseases. Secondary findings were seen in 5.4% of cases.

Conclusion: We present the data of a large cohort of pediatric patients from India diagnosed with neurogenetic disorders who displayed diversity in phenotypes and genotypes.

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P11.043.A CHAMP1 directs the maturation of synaptic function and firing activity in iPSC-derived excitatory neurons

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Background: Mutations in CHAMP1 are a major genetic risk factor for neurodevelopmental disorders (NDDs), with affected individuals exhibiting intellectual disability, autism spectrum disorder (ASD)-like behaviors, microcephaly, hypotonia, and distinctive dysmorphic features. CHAMP1 encodes for a zinc-finger protein involved in the chromosomal segregation during mitosis and in DNA repair mechanisms. However, the function of CHAMP1 in neurodevelopment remains relatively unexplored. We hypothesize that CHAMP1 orchestrates neuronal proliferation by modulating mitosis during prenatal development, ultimately promoting the maturation of synaptic and firing properties of excitatory neurons and synchronicity of local cortical circuits. CHAMP1 loss of function (LoF) could delay the maturation of both individual neurons and complex circuits.

Material and Methods: To test our hypothesis, we induced neural progenitor cells (NPCs) and mature neurons using a dual-SMAD inhibition approach with one neurotypical iPSC line (Ctrl1), two patient donor lines (CHAMP1(c.1489c>T), CHAMP1(c.2094-delT)), and one isogenic knockout line (iCHAMP1-/-).

Results: To quantify the development of functional properties in maturing neurons, we used whole-cell patch-clamp electrophysiology at one and two months in vitro. CHAMP1(c.1489c>T), CHAMP1(c.2094delT), and iCHAMP1-/- neurons exhibited altered membrane properties and reduced action potential amplitude and frequency compared to Ctrl1 neurons. Additionally, CHAMP1 LoF reduced the amplitude and frequency of spontaneous excitatory postsynaptic currents at 1 month in vitro.

Conclusion: More deficits were observed in CHAMP1(c.1489c>T) neurons compared to CHAMP1(c.2094delT), which correlates with the severity of CHAMP1 syndrome present in these patients. Together these results suggest that CHAMP1 directs the maturation of excitatory synaptic and firing activity.

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P11.044.C Characterising Epilepsy Phenotype Heterogeneity in Biobank-Derived Disease Cases

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Background: Epilepsy is a heterogeneous disorder with diverse genetic and clinical characteristics. This complicates genome-wide association studies (GWAS) on biobank data, where diagnoses rely on the International Classification of Diseases system rather than detailed clinical characterisation. We both explore the scope of this problem by estimating genetic correlations between biobank-derived and clinically validated samples, and whether family history can improve diagnostic accuracy.

Methods: We conducted a GWAS within the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) cohort (n = 4,409) using family history with LT-FH++. Genetic correlations between the biobank summary statistics - iPSYCH, FinnGen (n = 15,645), and UK Biobank (n = 3,500) - and the most recent International League Against Epilepsy (ILAE) GWAS of clinically validated samples (n = 29,944) were calculated using linkage disequilibrium score regression (LDSC).

Results: The biobanks were highly genetically correlated with the broadest ILAE GWAS phenotype (iPSYCH rg = 0.96, 95%CI 0.53–1.39, FinnGen rg = 0.78, 95%CI 0.59–0.97, UK Biobank rg = 0.84, 95%CI 0.49–1.19), suggesting consistent overall genetic architectures. Especially the UK Biobank summary statistics were highly genetically correlated with the genetic generalized subtype from the ILAE GWAS (rg = 0.92, 95%CI 0.56–1.27). Conversely, the focal ILAE GWAS subtypes resulted in higher estimates for iPSYCH (rg = 0.99, 95%CI 0.43–1.45) and FinnGen (rg = 0.74, 95%CI 0.45–1.03). Combing family history with the phenotype improved the iPSYCH-ILAE genetic correlations.

Conclusion: Non-validated biobank-derived epilepsy cases are highly genetically correlated with clinical cohorts, and combining family history with the phenotype can improve diagnostic accuracy. This improves the outlook of their inclusion in genetic meta-analyses.

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P11.045.D Transcriptomic signature of sporadic frontotemporal lobar degeneration with TDP-43 subtype C

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Background: Frontotemporal lobar degeneration (FTLD) with underlying TDP-43 subtype C pathology (FTLD-TDP-C) is a sporadic type of early-onset dementia with a homogeneous clinical presentation. The aim of this study was to discover dysregulated pathways in FTLD-TDP-C through transcriptomics of its most affected region: the left temporal cortex.

Methods and methods: Bulk RNA sequencing was conducted on temporal cortices of a post-mortem cohort of 18 FTLD-TDP-C and 23 controls. Differential expression and overrepresentation analyses were run to detect differentially expressed genes (DEG) and functionally annotate them. To identify DEG specific to FTLD-TDP-C, we removed genes also dysregulated in temporal cortices of other neurodegenerative diseases: Alzheimer's dementia, progressive supranuclear palsy and FTLD with *MAPT* 10 + 16 deletion. Using cell deconvolution, differences in cell type composition between cases and controls were corrected. Furthermore, we performed weighted gene co-expression network analysis (WGCNA).

Results: We found 6324 DEG with FDR < 0.05, of which 3073 were upregulated and enriched for immune response and cell structures, and 3251 were downregulated and enriched for neuronal structures and synapse activity. In 3352 DEG considered

specific to FTLD-TDP-C, RNA splicing was upregulated, whilst postsynaptic specialisation was downregulated. WGCNA identified 18 modules, of which five were significantly correlated to FTLD-TDP-C, and enriched for RNA splicing, postsynaptic function, non-neuronal damage response, and transmembrane transport over the blood-brain barrier.

Conclusion: In FTLD-TDP-C, one fourth of all genes were differentially expressed, and half of these were specific to FTLD-TDP. Differential expression analysis and WGCNA indicated that RNA splicing and postsynaptic processes are significantly dysregulated in FTLD-TDP-C.

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P11.046.A SPARK: a recontactable cohort of >150,000 people with autism engaged in research

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Background: SPARK is a cohort of autistic individuals and their family members that seeks to accelerate autism research.

Material and Methods: Participants are US residents who join online or through clinical sites and can choose to provide a saliva sample for exome- or whole-genome sequencing. SPARK collects medical history, family information and standardized questionnaires on the core symptoms of autism. SPARK returns genetic results to participants for autism/neurodevelopmental conditions with variants that meet American College of Medicine (ACMG) criteria for likely pathogenic (LP) or pathogenic (P).

Results: Exomes or genomes are available to approved researchers from nearly 58,000 participants (Table 1) with autism and 94,000 first-degree family members. Of sequenced participants with autism, 41% are from trios, 25% self-reported as people of color, 26% are primarily of non-European genetic ancestry (HapMap3), and 84% completed a medical history questionnaire. SPARK has returned 2,855 autism-related findings to participants and identified a LP/P variant in roughly 8.5 percent of autistic participants. The top 3 CNVs are 16p11.2 deletion, 16p11.2 duplication, and 22q11.2 duplication. The top 3 single nucleotide variants/indels are in *SCN2A*, *SHANK3* and *NF1*. Researchers can apply to recruit SPARK participants to their studies through SPARK's Research Match platform (base.sfari.org). Researchers have launched 304 studies using Research Match and engaged more than 50,000 SPARK participants.

Conclusion: SPARK provides a unique resource of recontactable autistic individuals and their families along with comprehensive genetic and phenotypic data that are freely available to approved researchers.

Characteristic (at enrollment)	Minors (N = 47,504)	Independent & Dependent Adults (N = 10,443)
Age at enrollment in years, mean (SD)	8.0 (4.3)	29.1 (11.1)
Sex at birth, N (%)		
Male	37,002 (77.9%)	5,960 (57.1%)
Female	10,502 (22.1%)	4,483 (42.9%)
Self-reported race, N (%)	**Children and adults (N = 49,443)**	
African American/Black	2,832 (5.7%)	
Asian	1,664 (3.4%)	
Native American/Native Hawaiian	315 (0.6%)	
White	37,052 (75.0%)	
Other	2,483 (5.0%)	
More than one race	5,097 (10.3%)	

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P11.048.C Biallelic loss of function variant in *SORL1* causes complex hereditary spastic paraplegia - from genomics to functional analysis

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Background: Genes regulating the endolysosome and autophagy pathway are the major aetiological contributors to hereditary spastic paraplegia (HSP). *SORL1*, an endosomal trafficking protein, is essential for maintaining the homeostasis of glutamatergic neurons and white matter tract integrity. It has so far only been associated with a dominant form of Alzheimer disease (AD) and amyloid angiopathy and its relationship with HSP through shared macroautophagy mechanisms remains unexplored.

Material and Methods: Whole exome sequencing using the KAPA HyperCap workflow (Roche Sequencing Solutions, USA) on DNBSEQ-G400 (MGI Tech, Latvia) platform with 100 bp paired-end reads was performed. Long-read whole genome sequencing (LR-WGS) library was prepared using the ligation sequencing kit (Oxford Nanopore Technologies, UK) and were sequenced on Promethion 2i. Functional validation included transcriptomic analysis and cellular studies using patient-derived fibroblasts.

Results: We identified a novel biallelic NM_003105.6:c.1211+1G>A consensus splice donor variant (ClinVar Accession: VCV003238954.1) in *SORL1*. Transcriptomics confirmed significant nonsense-mediated decay and aberrant splicing. The evidence of reduced levels of *SORLA* and, consequently, a significant enlargement of endosomes and lysosomes in patient-derived fibroblasts, substantiates the pathogenicity of the variant.

Conclusion: The constraint score metrics (pRec-1.0), meiotic recombination studies, *SORL1-/-*mice models displaying HSP-like phenotypes with cerebroretinal vasculopathy (CRV), and in vitro studies showing endolysosomal enlargement in *SORL1-/-* glutamatergic neurones all support the likelihood that *SORL1* is recessive and aetiologically related. Collating all the evidence from phenotype analysis, functional studies, and literature suggests, *SORL1* is probably a new candidate for a recessive form of complicated HSP, expanding its known role beyond AD-related pathologies.

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P11.049.D Gene Expression Profiling in Genetic Frontotemporal Dementia: Insights into Autophagy, Inflammation, and Aging

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Background: Frontotemporal dementia (FTD) is the leading cause of pre-senile dementia, characterized by behavioural or language impairments. Pathogenic mutations in GRN, MAPT, and C9orf72 are the most frequent genetic causes of FTD. We investigated the expression of 56 genes involved in autophagy, inflammation, and aging in FTD patients carrying these mutations, compared to sporadic FTD patients and healthy controls. While these pathways are central to FTD pathogenesis, their roles remain unclear.

Material and Methods: Total RNA was extracted from Paxgene-stabilized blood samples of 20 healthy controls, 10 C9orf72, 10 GRN, 10 MAPT mutation carriers, and 10 sporadic FTD patients. Real-time PCR analysis was performed using Custom TaqMan OpenArray technology, enabling simultaneous detection of 56 mRNAs. Gene expression levels were compared across groups using ANOVA with post hoc multiple comparisons.

Results: Several genes exhibited differential expression patterns among groups. Autophagy-related genes (ATG5, BECN1) were altered in mutation carriers, particularly C9orf72 carriers. Inflammation-associated genes (B2M, TYROBP) were deregulated in MAPT and C9orf72 carriers compared to sporadic FTD. NFE2L2, a key antioxidant and anti-inflammatory regulator, was significantly increased in MAPT but decreased in C9orf72 carriers, indicating differential oxidative stress regulation. SERPINB1 was upregulated in GRN carriers compared to controls.

Conclusion: Our findings support the involvement of autophagy, inflammation, and oxidative stress in FTD and highlight molecular differences between genetic and sporadic cases. These insights could help identify novel biomarkers and therapeutic targets for personalized interventions in FTD.

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P11.050.A De novo variant analysis of childhood-onset obsessive-compulsive disorder in the French-Canadian population

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Background: Childhood-onset obsessive-compulsive disorder (CO-OCD) is a neuropsychiatric disorder with a strong genetic component. De novo variants (DNVs) contribute to CO-OCD risk, but to date, no DNV analysis of CO-OCD has been performed in a genetically isolated population. Here, we investigated the impact of rare de novo single nucleotide variants (dnSNVs) on CO-OCD risk in the French-Canadian population.

Material and Methods: Whole-exome sequencing was performed on 42 trios ($n = 126$). Variants were filtered for heterozygous genotype in the proband and wildtype in both parents. Protein-coding dnSNVs with minor allele frequencies <1% were retained. DNV rate (μ) was calculated as: μ = (number of observed dnSNVs/proband)/(size of RefSeq hg19 coding exome x number of gametogeneses). DenovolyzeR assessed the genome-wide burden of dnSNVs by variant class. Genes harbouring dnSNVs were examined for overlap with genes previously associated with OCD and other psychiatric disorders. Our candidate gene list was combined with those from previous CO-OCD DNV studies for functional enrichment meta-analyses.

Results: 34 dnSNVs in 34 genes were observed across 36 probands (0.94 dnSNVs/proband, average CADD = 16.1). μ was 1.40E-08 per nucleotide per generation (95%CI:9.36E-09,1.86E-08). A nominal enrichment of splice-site dnSNVs was detected ($p\text{-}adj = 0.0519$). Four genes were previously associated with OCD. All 34 genes overlapped with genes associated with 11 related neuropsychiatric disorders. Genes involved in clathrin-dependent endocytosis (GO:0072583) and the fetal brain were overrepresented in the combined gene list ($p\text{-}adj < 0.05$).

Conclusion: This study provides the first comprehensive list of candidate CO-OCD genes specific to the French-Canadian population, offering a framework for DNV analyses in isolated populations.

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P11.051.B Genome-wide copy number variation association study in schizophrenia

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Background: Copy number variations (CNVs) are a class of structural variations that have been linked to a number of psychiatric disorders, including schizophrenia spectrum disorder (SSD). Although genomic studies have successfully identified (common) risk variants in SSD, the rarity of CNVs in the general population, the complexity of CNV detection, and the focus on a limited number of known and well-studied CNVs have limited the scope of previous studies and restricted more comprehensive analysis of CNVs in SSD.

Material and Methods: This study aims to perform a comprehensive genome-wide CNV enrichment analysis, utilizing microarray data from the iPSYCH2015 case-cohort sample, which includes 13,147 SSD cases and 43,177 population controls. Specifically, the genome-wide burden of recurrent and non-recurrent CNVs in SSD will be assessed, as well as the overall CNV heritability. Furthermore, we will investigate the genome-wide association of CNVs in SSD in the Danish population and examine CNV enrichment in defined brain-expressed gene sets to provide functional insights.

Results: : Preliminary results suggest a higher overall burden of CNVs in brain-expressed genes among individuals with SSD compared to population controls, and that their aggregation in specific gene networks may provide insights into the genetic etiology of SSD.

Conclusion: Our findings will enhance the understanding of the contribution of CNVs to SSD, leading to better estimates of individual risk and establishing a foundation of future studies.

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P11.052.C Polygenic scores for dyslexia are associated with lower general cognitive ability and reduced accuracy in UK Biobank participants with psychiatric disorders

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Background: Polygenic scores (PGS) for dyslexia have shown associations with cognitive skills, and psychiatric disorders share genetic overlaps with cognitive dimensions. This study investigated whether dyslexia PGS (i) correlate with PGS of cognitive and psychiatric phenotypes, and (ii) predict cognitive profiles in patients with psychiatric disorders.

Methods: PGS for schizophrenia (SCZ), bipolar disorder (BD), major depressive disorder (MDD), dyslexia (DYX, based on 23andMe, Inc. GWAS), word reading (WR), and educational attainment (EA) were calculated using PRS-CS and correlated with cognitive measures from 406,842 UK Biobank participants after genetic quality control. Using confirmatory factor analysis, we modeled 12 measures from seven cognitive categories through a bifactor model, revealing a general cognitive factor with specific speed and accuracy components. Additionally, the association between DYX PGS and cognitive factors was tested in a subset of 28,666 individuals with mental disorders based on ICD-10 F-codes.

Results: Positive significant ($p < 0.001$) correlations were observed between DYX PGS and psychiatric PGS (SCZ, BD, MDD; $r = 0.02–0.06$). The bifactor model displayed good fit indices (CFI = 0.99, RMSEA = 0.027). In patients with psychiatric disorders, DYX PGS was significantly associated with lower general cognitive ability ($\beta = -0.013$, padj = 0.01), accuracy ($\beta = -0.021$, padj < 0.001), and alphanumeric trail-making speed ($\beta = -0.071$, padj < 0.001). The strongest associations regarding accuracy were between DYX PGS and fluid intelligence ($\beta = -0.104$, padj < 0.001) and numeric memory ($\beta = -0.122$, padj < 0.001).

Conclusions: Polygenic risk for dyslexia predicted lower cognitive performance in patients with psychiatric disorders, particularly for general ability, accuracy, and processing speed. Genetic overlap between dyslexia and psychiatric risk may partly explain comorbidities.

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P11.053.D Biallelic missense variant c.497G>A in *PTPN23* is associated with hereditary spastic paraplegia

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Background: Biallelic variants in *PTPN23* are associated with neurodevelopmental disorders and structural brain anomalies, with or without seizures and spasticity (NEDBASS, MIM #618890), a severe early-onset disorder.

Material and Methods: Clinical evaluation of the proband and her affected sibling was performed. Singleton exome sequencing (ES) was done for the proband, followed by Sanger validation and segregation in the family. Subsequently, in silico mutagenesis analysis was performed.

Results: A clinical diagnosis of hereditary spastic paraplegia (HSP) was considered in a 23-years-old female and her brother with progressive difficulty in walking and hyperreflexia of lower limbs. ES identified a rare homozygous missense variant c.497G>A p.(Ser166Asn) in *PTPN23* (NM_015466.4). Sanger sequencing showed the variant in homozygous state in both siblings with parents as carriers. Multiple in silico prediction tools predicted the variant to be damaging to the protein function. This variant is present in the BRO1 domain of PTPN23 protein, an essential binding site for the formation of multivesicular bodies. In silico mutagenesis analysis showed altered interactions with its neighbouring residues in the mutant protein compared to wild-type. Twelve individuals from 12 unrelated families were reported with NEDBASS had missense and frameshift variants situated across all domains of PTPN23, however, recently one family with six individuals presenting with HSP and microcephaly had a biallelic frameshift variant in the C-terminal region of PTPN23.

Conclusion: We herein report a family with HSP harbouring a biallelic missense variant in *PTPN23*. Further functional studies are underway to establish the consequences of the variant using patient fibroblasts.

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P11.054.A *FLNA*Genomic Rearrangements in a 391 French Bilateral Periventricular Nodular Heterotopia Cohort: Prevalence and Phenotypic Correlations

Henri MARGOT[1], *Chloé ANGELINI*[2], *Benoit Arveiler*[2], *Caroline ROORYCK THAMBO*[2], *Cyril GOIZET*[2], *Patricia FERGELOT*[2]

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Background: *FLNA* loss of function manifests across a broad spectrum of phenotypes, ranging from severe prenatal onset to asymptomatic cases. Bilateral periventricular nodular heterotopia (BPNH) consistently occurs in affected individuals. This retrospective study involving French patients with BPNH evaluates the prevalence of *FLNA* gene dosage anomalies and investigates genotype-phenotype correlations in a large cohort of French patients with BPNH.

Methods: A retrospective observational study was conducted on 391 individuals diagnosed with BPNH confirmed by brain MRI. Sequencing analysis using Sanger or Next-Generation Sequencing was complemented by targeted array-comparative genomic hybridization to identify CNVs.

Results: *FLNA* variants were identified in 40% of females and 12% of males. Among these, 87% were SNVs, while CNVs accounted for 13%, all of which were deletions. Half of the CNVs involved a recurrent deletion spanning exons 31–48, often accompanied by a duplication of the neighboring *EMD* gene. This del-dup was associated with a milder phenotype, whereas smaller de novo deletions correlated with severe outcomes. Mosaicism was also detected in 3 cases.

Conclusion: *FLNA* CNV analysis, particularly for recurrent deletions and mosaicism, is essential in the genetic evaluation of BPNH. Integrating CNV detection with SNV analysis improves diagnostic accuracy and enhances understanding of genotype-phenotype correlations.

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P11.055.B *POLG* variants in Parkinson's disease: a pilot study

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Background: *POLG* variants are associated with variable clinical manifestations, including extrapyramidal syndrome, and have been rarely reported in patients with Parkinson's disease (PD). The aim of the study was to establish the frequency of POLG variants in patients with PD.

Material and Methods: PD subjects and patients with other neurological disorders (non-PD) consecutively underwent genetic analysis. Exomes from unrelated Italian healthy controls were obtained from the Network for Italian Genomes (NIG-ExIT) cohort. Only *POLG* variants with a Combined Annotation Dependent Depletion (CADD) score more than 20 and classified as variant-of-unknown-significance (VUS), likely pathogenic, or pathogenic, were considered.

Results: Genomic data from 982 PD patients, 1080 non-PD patients, and 1686 healthy controls were analyzed. *POLG* variants were identified in 26 PD (2.65%), 21 non-PD (1.94%), and 39 healthy subjects (2.31%). A total of 48 *POLG* variants were detected, of which 15 (31.25%) were found only in PD patients. According to the chi-square test, no significant differences in *POLG* frequencies were found between PD and non-PD patients ($p = 0.29$) or healthy controls ($p = 0.96$).

Conclusion: No significant differences in *POLG* frequencies were detected between PD and non-PD patients or healthy subjects. However, several rare *POLG* variants were identified only in PD patients. The role of *POLG* in PD pathogenesis remains unclear. Case-control studies are limited due to the clinical heterogeneity of *POLG* and its possible onset in advanced age. Further studies with larger cohorts are needed to better understand the role of *POLG* in PD.

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P11.056.C Long-read sequencing reveals an unexpected complexity of repeat architecture in *C9orf72*/FTDALS1 families

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Background: Repeat expansion (RE) in *C9orf72* gene is the most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Systematic definition of *C9orf72* RE size and sequence in a clinical settings it crucial for precise genetic definition, making it possible to discover clinicopathological associations, and to improve genetic counselling and patient stratification. The aim of this study is to exploit long-read sequencing (LRseq) technologies to precisely define length and architecture of *C9orf72* RE in a clinical setting.

Material and Methods: *C9orf72* expanded region was analyzed in 11 subjects (3 sporadic patients, 6 familial cases, and 2 presymptomatic probands) with both Southern blot (SB) and LRseq with Oxford Nanopore Technologies (ONT) and PacBio HiFi sequencing.

Results: Size mosaicism of expanded allele was estimate by SB (61-4659), ONT (38-3878) and PacBio (53-4412), with a good correlation up to 1500 repeats. Significant intergenerational variability was observed in one family that exhibits allele contraction from an affected father to his asymptomatic daughter. Interestingly, both ONT and PacBio LRseq revealed the presence of mosaic repeat interruptions in expanded alleles. Moreover, PacBio LRseq allowed us to identify a novel alternative motif allele $((G_4C_2)(CG_3CG))_n(G_4C_2)_n$, proving to be crucial for the resolution of cases in which Repeat Primed-PCR approach fails to define the presence of the expansion.

Conclusion: These results demonstrate the essential contribution of LRseq approaches to the understanding of the variability and structure of repeat regions as well as their intergenerational and somatic instability, with crucial implications for diagnosis and pathogenic studies.

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P11.057.D Novel *UBA7* gene variants disrupt ISGylation and are associated with neurodevelopmental disorders

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Background: UBA7 is an enzyme belonging to the family of E1-ubiquitin activating enzymes. It is involved in the activation of ubiquitin-like protein ISG15, which then leads to a ubiquitin-like enzymatic cascade called ISGylation. While UBA7 and ISGylation are involved in various disease processes, such as cancer and neurodegenerative disease, so far no disease-causing genetic variants have been described. Here we identify the first instance of genetic *UBA7* variants presenting with neurodevelopmental disorders, and provide functional data on expression and enzymatic function.

Methods: Through international cooperation, three individuals carrying *UBA7* variants were identified. Genetic variants were identified through exome sequencing. Functional analyses of variants, including ISGylation activity assays and protein and transcript expression, were performed.

Results: Individual A presented with intellectual disability/global developmental delay, early onset seizures, repetitive

behaviour, mild microcephaly and recurrent infections. Individual B presented with intellectual disability/developmental delay, autism and obesity. Individual C presented with severe epilepsy and regression, and passed away at age three. The genetic variants identified in *UBA7* were, respectively, a homozygous c.933G>A; p.W311*, a homozygous c. 2126_2129delAACA; p.Lys709Serfs*45 and a homozygous c.1642G>T; p.Val548Leu. Functional analyses showed decreased protein and transcript expression of the Lys709Serfs*45 variant, as well as decreased ISGylation activity. The Val548Leu variant showed increased ISGylation activity.

Conclusion: This study is the first to identify and provide functional data on novel variants in *UBA7* associated with neurodevelopmental disorders. Our results show the potential importance of UBA7 and ISGylation in neurodevelopment, and identify a new gene to be considered in future diagnostics.

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P11.058.A Per-domain rare variant burden analysis in 150 Parkinson's risk genes reveals novel functional targets

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Background: Numerous genes have been associated with Parkinson's Disease (PD), and many more have been nominated as potentially associated based on a combination of genome-wide association and machine learning (ML) studies. In this study, we perform a per-domain rare variant burden analysis across PD-associated or -nominated genes to uncover novel, functionally specific associations.

Material and Methods: We meta-analyzed 150 PD risk-associated/ML-nominated genes for per-domain rare variant burden (minor allele frequency ≤0.01) using optimized kernel association test (SKAT-O). The study population consisted of UK Biobank (UKBB) and Accelerating Medicines Partnership program for Parkinson's disease (AMP-PD) cohorts (Table 1). The analysis was conducted separately with and without proxy cases. Variants were grouped by domain and stratified into exonic, non-synonymous, loss-of-function, and CADD>20 categories, applying false discovery rate corrections.

Results: Post-corrections, without proxies, we only observed statistically significant rare variant burden in the *LRRK2* kinase domain in UKBB ($P_{exonic} = 0.003$; *Pnon-synonymous* $= 0.003$; $P_{CADD\text{-}score>20} = 0.003$) and in the meta-analysis ($P_{exonic} = 0.002$; $P_{non\text{-}synonymous} = 0.002$; $P_{CADD\text{-}score>20} = 0.002$). In the meta-analysis, including proxies from UKBB cohort, domain-level strong novel post-correction associations emerged in three genes: *PITX3* (OAR domain) ($P_{exonic} = 1.22 \times 10^{-6}$; $P_{non\text{-}synonymous} = 1.28 \times 10^{-7}$), *SATB1* (NLS) ($P_{CADD\text{-}score>20} = 3.66 \times 10^{-3}$), and *POLR2A* (RNA_pol_Rpb1_R) ($P_{CADD\text{-}score>20} = 7.51 \times 10^{-3}$).

Conclusion: *LRRK2* associations remain robust. Incorporating proxy cases revealed novel domain-specific signals in *PITX3*, *SATB1*, and *POLR2A*, nominating potentially valuable therapeutic targets for PD and warranting further functional investigation.

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P11.059.B Moyamoya angiopathy in pediatric European patients: novel candidate genes and their associated biological roles

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Background: Moyamoya angiopathy (MMA) is a rare condition characterized by stenosis of the intracranial internal carotid artery and its branches resulting in transient ischemic attacks and strokes. Previous studies, mainly focused on adults and/or Asians, have suggested several susceptibility genes, with *RNF213* variant p.R4810K being the only recurrent. In adult European MMA patients, diverse *RNF213* variants have been positively associated, but not p.R4810K.

Material and Methods: Exome sequencing was performed from blood samples in a pediatric cohort of 104 MMA patients and 520 healthy controls. We performed a rare-variant enrichment test using the R package SKAT and a trio analysis of 62 patients. We repeated the test with a sub-cohort of 80 cases and 381 controls predicted as 100% of European origin.

Results: Both tests identified a significant enrichment of rare variants in genes previously associated with MMA, *RNF213* and *NF1*, including several de novo variants. When interrogating the full cohort, 24 genes are significantly enriched, six of them being novel and common in both tests. Gene Ontology analysis unraveled their association in diverse biological activities, including angiogenesis, endothelial, developmental, and neurobiological processes. Among the top enriched genes was *COL18A1*, for which one patient even showed a de novo variant. *COL18A1* is located on chromosome 21 and thus might be involved in the increased MMA risk of Trisomy 21 patients.

Conclusion: We present an international MMA case-control study focusing exclusively on pediatric patients, predominantly of European origin. The results show novel candidate genes that could advance the understanding of MMA.

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P11.060.C Network analysis for gene discovery in ataxia: exploring antibody targets

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Background: Recent discoveries have identified novel autoimmune diseases manifesting as neurological movement disorders such as ataxia. Some target proteins of these antineuronal antibodies are encoded by genes associated with ataxia,

presenting an opportunity to uncover new ataxia-related genes through a network analysis with known antibody targets as primary seeds.

Methods: We performed a network analysis using genes encoding epitopes targeted by known antineuronal antibodies associated with ataxia. Protein-protein interaction data from the STRING database were analyzed to identify candidate genes directly interacting with known ataxia genes. Genes with the strongest connections to these ataxia genes were prioritized as potential novel monogenic causes of ataxia.

Results: From a combined list of ~80 antibody target genes and ~300 known movement disorder genes related to ataxia, we identified 64 novel potential candidate genes for ataxia, including 20 known antibody target genes. Currently, we are analyzing >1,500 exome/genome datasets from previously undiagnosed patients and families with ataxia retrieved from Solve-RD and Radboudumc to identify rare variants in the genes identified through this network analysis. Amongst 2,000 rare SNVs in Solve-RD's cohort of unsolved ataxia cases, filtering and prioritization is ongoing, considering e.g. inheritance mode, recurrence, and variant-effect predictions.

Conclusion: Our network analysis has identified 64 new potential candidate genes for ataxia. Ongoing analyses of rare variants in these genes, conducted within cohorts with available exomes and datasets from patients with suspected monogenic forms of ataxia, will provide information on the actual potential of this approach.

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P11.061.D Pathway polygenic risk scores analysis in an East Asian treatment resistant scizophrenia cohort

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Background: Schizophrenia is a highly heritable disease with typical onset during adolescent and early adulthood. Around 25% of schizophrenia patients eventually develop treatment-resistant schizophrenia (TRS), a severe type associated with poor prognosis and response to non-clozapine antipsychotics. Neuroimaging evidence suggested that TRS patients have less striatal presynaptic dopamine synthesis capacity than non-TRS patients, which is highly interconnected with the synaptic functions. This study aims to clarify whether TRS and non-TRS patients would differ in synaptic pathways using genetic data.

Material and Methods: We performed whole genome sequencing in a 407 schizophrenia patients cohort recruited from Castle Peak Hospital in Hong Kong between 2014–2018. As of 2022, 142 were having TRS. To test the hypothesis that TRS and non-TRS patients could differ in brain synaptic pathways, we calculated pathway polygenic risk scores (pPGSs) using expert curated pathway gene sets related to synapses. Competitive and non-competitive tests were performed with software PRSet, to estimate the level of associations and enrichments.

Results: We found one pathway, extracellular matrix of synaptic cleft, reached the significance level after Bonferroni-correction ($p < 1 \times 10^{-4}$) in both competitive and non-competitive tests. An associated quantile plot suggested significant differences in extracellular matrix of synaptic cleft among those who carried the top 20th and bottom 20th percentiles of the associated pPGSs.

Conclusion: Our pPGSs findings with synapses gene sets implicates the involvement of extracellular matrix of synaptic cleft in TRS; these gene sets are more enriched than the rest of the genome in TRS patients.

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P11.062.A Epigenetic markers linked to early-onset schizophrenia in the Chinese population

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Background: Age at onset (AAO) is a key phenotype in schizophrenia. Early-onset schizophrenia (EOS; AAO < 18) is linked to greater neurological and neurodevelopmental impairments and a poorer prognosis compared to adult-onset schizophrenia (AOS). While most genetic research on AAO and EOS has focused on candidate genes and genome-wide association studies (GWAS), the role of DNA methylation, an epigenetic mechanism influenced by both environmental and genetic factors, remains less extensively studied in the context of EOS and AAO.

Methods: Peripheral blood DNA samples were collected from 120 Chinese patients diagnosed with schizophrenia, with a mean AAO of 15 for the 49 EOS cases and 36 for the 71 AOS cases. The Illumina Infinium Human Methylation EPIC v2.0 BeadChip was utilized, and an epigenome-wide association study (EWAS) was performed to compare methylation patterns between EOS and AOS groups, with adjustments for age, sex, smoking status, and blood cell composition.

Results: We identified 49 differentially methylated positions (DMPs) for EOS that achieved a suggestive significance level (p-value < 5e-05). Several genes annotated from these DMPs, including *ORMDL1*, *ANXA4*, and *TRRAP*, were located in genomic regions associated with schizophrenia or EOS. Additionally, we discovered novel regions linked to cognitive performance, including *AKAP8L*, *C4orf45*, and *GTF2I*. Enrichment analysis suggested that processes involving kinase activity, cell cycle regulation, and microRNAs in cancer may relate to EOS.

Conclusion: This research provides new insights into the distinct DNA methylation patterns linked to EOS in the Chinese population and highlights several potential biological pathways involved in the condition.

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P11.063.B Exploring Parkinson's disease pathogenesis using a multi-omics approach: insights from DNA methylation and circular RNA profiles in discordant monozygotic twins

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Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic neurons loss in the substantia nigra. While both genetic and environmental factors contribute to PD, its etiology remains unclear. This study investigates unknown factors contributing to PD using a unique cohort of 30 monozygotic (MZ) twins discordant for the disorder through a multi-omics approach including transcriptome, circular RNA (circRNA), and methylome profiling.

Material and Methods: NGS libraries were prepared starting from DNA and RNA extracted from blood samples of MZ twins. DNA methylation was analyzed with the Bismark Bisulfite Mapper software and the MethylKit R package, while DCC and DESeq2 were used for circRNA and transcriptomics analyses.

Results: We detected 471 differentially methylated positions, with 169 showing ≥8% differential methylation. Among these, the top hypermethylated region (+22%, q = 6.96E-27) locates in the *MTF1* intron 2. This gene plays a potential neuroprotective role against oxidative stress. Among the 2,307 circRNAs identified, 40 were deregulated in PD twins. Notably, a circRNA -derived from exons 2–4 of *SNCA* (circSNCA)- exhibited reduced expression in affected twins compared to both their healthy counterparts (log2FC = −0.545, p = 0.024) and an unrelated control cohort (log2FC = −1.493, p = 0.024).

Conclusion: The identification of differential methylation in *MTF1*, a gene with potential neuroprotective functions, and the deregulation of circSNCA, a key PD-related circRNA, could highlights novel biomarkers that may drive PD pathogenesis. In the future, we plan to integrate all the omics data collected for this cohort to enhance the overall understanding and gain deeper insights into the molecular mechanisms underlying PD.

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P11.064.C Uncovering hidden causes in developmental epileptic encephalopathy: high resolution structural variant detection with optical genome mapping

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Background: Despite the use of standard genetic diagnostic methods, the underlying cause remains unidentified in a significant proportion of developmental epileptic encephalopathy (DEE) cases. Optical genome mapping (OGM) is a novel technology that enables high-resolution detection of structural variants (SVs). This study aimed to evaluate the diagnostic contribution of OGM in DEE cases with unresolved genetic causes.

Material and Methods: The study included 38 DEE cases, aged 0–18 years, who remained undiagnosed despite conventional karyotyping, chromosomal microarray analysis, and whole-exome sequencing (WES). Patients were reassessed based on clinical, electrophysiological, biochemical, and radiological criteria. Genetic data of eligible patients were reanalyzed, and OGM was applied to cases with no identified etiology. Potential findings from OGM were validated using appropriate laboratory techniques.

Results: Reanalysis of WES data identified two pathogenic variants in two cases (5.2%): SCN2A (c.221T>C, p.Val74Ala) and WDR45 (c.827+5G>A), leading to definitive molecular diagnoses. OGM was performed on the remaining 32 cases, detecting five potentially pathogenic SVs, of which three were excluded after validation. Two SVs (6.3%) were confirmed and corresponded to the following molecular diagnoses: Fragile X syndrome [ogm[GRCh38] ins(X;?)(q27.3;?)] and PHACTR1-related DEE [ogm[GRCh38] 6p24.1(13083829_13279761)x1].

Conclusion: Reanalysis processes and OGM improved the diagnostic success rate in DEE cases with unresolved genetic causes. OGM provides significant diagnostic contributions by identifying high-resolution SVs. When standard methods are insufficient, genomic data reanalysis and innovative techniques like OGM offer promising pathways for discovering rare variants and refining diagnostic strategies.

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P11.065.D 5mC and 5hmC detection highlights how psychedelics affect brain epigenetic patterns

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Background: Psilocybin, a serotonergic psychedelic compound, has demonstrated long-term antidepressant effects, yet its underlying mechanisms remain unclear. Emerging evidence suggests that epigenetic modifications, particularly 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), may play a role in its therapeutic action. Unlike passive demethylation, which requires cell division and occurs infrequently in neurons, active demethylation via TET enzymes rapidly converts 5mC to 5hmC, a process abundant in the brain. This study investigates changes in 5mC and 5hmC levels in key brain regions following psilocybin treatment.

Material and Methods: Male C57BL/6 mice were divided into two groups: psilocybin-treated (n = 3) and saline-treated control (n = 3). After 72 hours post-treatment, prefrontal cortex (PFC) tissue was collected for DNA methylation analysis using nanopore sequencing, enabling genome-wide detection of 5mC and 5hmC at single-base resolution.

Results: A total of 1,404 DhMRs were identified in psilocybin-treated mice, showing significant enrichment in the glutamatergic synapse pathway (q = 0.0009). In contrast, only 51 significant DMRs were found for 5mC, with no pathway enrichment detected. These findings suggest that 5hmC modifications, rather than 5mC, play a critical role in psilocybin's effects on synaptic plasticity and neuroplasticity.

Conclusion: Preliminary findings suggest that psilocybin induces rapid epigenetic changes, primarily via 5hmC, in key brain regions involved in mood regulation. These changes may underlie the sustained antidepressant effects of psilocybin, potentially offering new therapeutic targets for Major Depressive

Disorder (MDD). Future studies will focus on validating these results and exploring their long-term implications.

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U. Bertocchi: None.

P11.066.A Pathogenicity of missense neurogenetic variants: a focus on the NMDA receptor subtypes

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Background: Identification of variants, particularly in genes encoding the subunits of NMDA receptors (NMDARs), mediating gulutamatergic signals, is crucial for diagnosing diseases linked to NMDAR channelopathies, including epilepsy. Computational prediction of variant pathogenicity is important, but the performance of predictive models in this specific gene context remains underexplored. This study evaluates state-of-the-art models against neural network-based AlphaMissense for the classification of missense variants in genes encoding NMDAR subtypes.

Material and Methods: Pathogenic and benign missense variants in GRIN1, GRIN2A, and GRIN2B (GRIN genes) encoding the subunits of NMDAR subtypes were retrieved from the ClinVar database and classified using AlphaMissense, REVEL, BayesDel, ClinPred, PrimateAI, and CADD. Accuracy metrics were calculated using a custom Python script. Additionally, AlphaMissense performance was analyzed across NMDAR subunit domains. Statistical significance of performance differences was assessed using Fisher's exact test. Correlation of AlphaMissense scores with variant functional impact obtained from the literature was also evaluated by calculation of Spearman's Correlation Coefficient.

Results: AlphaMissense showed superior performance for variant classification compared to the other predictive models (Table 1). NMDAR subunit domain-based variant classification of AlphaMissense highlighted performance differences across different regions ($p < 0.05$). Moderate correlation between AlphaMissense scores and variant functional impact was found especially for cell surface expression.

Conclusion: Our findings contribute to the understanding of the variant effect of the GRIN genes, bottleneck in bridging the gap between genetic findings and the development of personalized treatment strategies for neurogenetic diseases linked to NMDAR channelopathies.

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P11.068.C Remodelling of neuronal RNA metabolism during Prader-Willi syndrome

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Background: Deletions in the paternally imprinted genomic region at 15q11.2-q13 that include two human C/D box small nucleolar RNAs (snoRNAs), SNORD115 and SNORD116, cause the neurodevelopmental disorder Prader-Willi syndrome (PWS). Both snoRNAs are strongly accumulated during neurodevelopment. Canonical box C/D snoRNAs carry the RNA methyltransferase Fibrillarin (FBL). During neuronal differentiation, FBL expression decreases. In contrast we saw a dramatic increase in expression of FBLL1; a largely uncharacterized, 83% identical, FBL homolog. FBL is required for the stabilization of small nucleolar ribonucleoprotein (snoRNPs) and methylation of target RNAs. We hypothesized that FBLL1 has similar functions, specifically in neuronal cells.

Material and Methods: Transcriptome changes upon FBLL1 deletion were identified using small RNA sequencing and total RNA sequencing. Pulldowns followed by mass spectrometry will determine protein binding partners. Small RNA sequencing in strains lacking SNORD115 or SNORD116 assessed the roles of these snoRNAs.

Results: FBLL1 is not required for neuronal stabilization of SNORD115 or SNORD116; we are determining effects on other snoRNAs. Cells lacking either SNORD115 or SNORD116 showed altered levels of several other ncRNAs. The multiple tandem snoRNA repeats in these clusters are related, but non-identical, and differ in relative accumulation.

Conclusion: Increased expression of FBLL1 during neuronal differentiation suggests functions related to this process. However, FBLL1 does not stabilize SNORD115 or SNORD116. We are testing the effects of FBLL1 gene knockout on the transcriptome during neuronal differentiation, and identifying protein and RNA binding partners. Links between RNAs altered by loss of SNORD115 or SNORD116 and PWS phenotypes are being tested.

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P11.069.D *FGF14*$^{(GAA)n}$-related ataxia: solving old cases, creating new challenges

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Table 1. Accuracy metrics of predictive models for the variant classification of the GRIN genes

	MCC(GRIN1)	MCC(GRIN2A)	MCC(GRIN2B)	MCC(ALL)	AUC ROC(GRIN1)	AUC ROC(GRIN2A)	AUC ROC(GRIN2B)	AUC ROC(ALL)	PR ROC(GRIN1)	PR AUC(GRIN2A	PR AUC(GRIN2B)	PR AUC(ALL)
AlphaMissense	0.61	0.77	0.81	0.78	0.87	0.97	0.97	0.96	0.92	0.96	0.98	0.96
BayesDel	0.29	0.67	0.63	0.58	0.76	0.91	0.91	0.88	0.84	0.85	0.93	0.86
CADD	0.18	0.4	0.43	0.41	0.84	0.88	0.87	0.88	0.9	0.75	0.84	0.82
ClinPred	0.46	0.52	0.5	0.52	0.87	0.97	0.92	0.93	0.92	0.94	0.93	0.92
PrimateAI	0.54	0.68	0.65	0.67	0.81	0.92	0.91	0.9	0.89	0.88	0.92	0.87
REVEL	0.26	0.57	N/A	0.49	0.75	0.91	N/A	0.88	0.84	0.82	N/A	0.82

Salsano[3], Davide Pareyson[3], Cinzia Gellera[1], Mariotti Caterina[2], Franco Taroni[1]

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Background: SCA27B is a common cause of dominant adult-onset spinocerebellar ataxia due to an intronic heterozygous GAA repeat expansion in the *FGF14* gene. Uninterrupted expansions ≥300 GAA are considered pathogenic, while uninterrupted expansions ≥250 exhibit incomplete penetrance. The role of the expansion between 180 and 250 GAA is still debated.

Material and Methods: The *FGF14* expansion was analysed by long-range PCR, bidirectional fluorescent RP-PCR, and LongRead-sequencing to 1) screen for the FGF14 expansion a cohort of Italian patients with ataxia (n = 404) or episodic ataxia (EA) (n = 88); 2) identify the exact architecture of the repeated sequence; 3) evaluate the threshold of pathogenicity.

Results: Expansions ≥250 GAA were identified in 25% of EA patients and in 7.5% of patients with chronic-progressive ataxia. Interestingly, the frequency rises to 80% in a subset of patients presenting with down-beating nystagmus, that was effectively treated with 4-aminopyridine. In both patients and controls, approximately 1% of alleles have an alternative/interrupted expanded repeat configuration. Furthermore, 1% of patients with ataxia present 1 or even 2 expanded alleles in the range 180–250. In these cases, LongRead-sequencing allows the precise definition of allele size and sequence, suggesting a wider range of pathogenic expansions.

Conclusions: *FGF14* expansion accounts for up to 25% of Italian non-EA1/non-EA2 families, presenting with late-onset EA characterized by disequilibrium, eye movement abnormalities, followed by a slowly chronic-progressive ataxia. LongRead-sequencing is crucial to determine the pattern and the size of the uncertain expanded alleles and to estimate the pathogenic threshold of the repeat expansion.

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P11.070.A Description of the clinical phenotype of metachromatic leukodystrophy in la reunion island due to a new variation with a founder effect

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Background: Metachromatic leukodystrophy is a rare recessive autosomic neurodegenerative disorder, insufficiently known and misdiagnosed. Four main phenotypes defined by the age at onset, have been described. Nowadays, there is a avaible curative treatment by gene therapy, to propose to MLD pre-symptomatic and early symptomatic patients only. Early diagnosis is therefore crucial. In the population of La Reunion island we have found 2 molecular variations wich are link to the disease and not found abroad. Maybe it's due to a founder effect, because it's an isolated entity. The aim of this study was to describe the natural history of the presentation of MLD in La Reunion island, and to investigate the process that led to final diagnosis in patients.

Material and Methods: Monocentric, retrospective study based on the description of clinical data of pediatric MLD patients, followed within the hospital of La Réunion.

Results: 15 patients were included to study epidemiological features, 12 to analyze the initial medical presentation leading to diagnosis and 12 to describe the clinical evolution. All the patients were diagnosed with the both same molecular variations wich are from La Reunion and not found abroad.

Conclusion: This study brings a better understanding of the natural history and the initial clinical presentation of MLD in the population of La Reunion island. It emphasizes the importance to raise awareness of the disease existence, and how to identify its alarms signs. We would like to propose a preconception diagnosis to patients from La Reunion island.

C.O.I. Disclosure:

A.D. Calaya: None.

P11.071.B Findings from single center exome sequencing in pediatric autism: an analysis of genetic variants and clinical correlations

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Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition marked by difficulties in social interaction and communication, along with restricted and repetitive behaviors. Affecting approximately 1 in 100 children, ASD has a strong genetic component. This study aimed to investigate the relationship between genetic variants identified in pediatric ASD patients and their clinical manifestations to enhance the understanding of ASD's genetic basis.

Material and Methods: Exome sequencing (ES) was performed on these patients from whom chromosomal abnormalities and Fragile X syndrome were excluded previously.

Results: The study involved 90 pediatric patients diagnosed with ASD (aged under 18), who were categorized into isolated (54 patients: 39 males and 15 females) and syndromic ASD groups (36 patients: 24 males and 12 females). In the isolated group, exome sequencing detected pathogenic variants in 16 individuals (29.6%), with a diagnostic yield of 30.7% in males and 26.6% in females. The syndromic group, which presented additional conditions like epilepsy and brain anomalies, showed a higher diagnostic yield, with 21 patients (58.3%) found to have pathogenic variants.

Conclusion: Our findings highlight the genetic heterogeneity of ASD, demonstrating that genes involved in transcriptional regulation, chromatin remodeling, and neurogenesis contribute to its pathogenesis, with key genes such as *MECP2, PTEN, EP300*, and *AGAP1* implicated in its development. Genetic factors were found to be twice as influential in syndromic ASD cases. These results support the use of ES as a primary diagnostic approach for understanding the genetic complexity of ASD.

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P11.072.C Gene Network-Based Partitioned Polygenic Risk Scores Reveal Clinical Insights into Schizophrenia

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Background: Schizophrenia is a severe mental disorder. Many genetic variants have been identified as risk factors for developing the condition, but translating these insights into actionable biological mechanisms remains a challenge. Gene networks defined by cell type-specific protein-protein interaction partners of high-risk schizophrenia variants have been proposed to be enriched in biologically relevant pathways related to schizophrenia. We propose a framework to prioritize genetic variants by assessing whether polygenic risk scores (PRS) partitioned to genetic variants in such networks are associated with clinical features of schizophrenia.

Material and Methods: Using genotypes from approximately 14,000 Schizophrenia Spectrum Disorder patients in the Danish iPSYCH cohort, in combination with publicly available summary statistics, we calculate PRS partitioned to gene networks. We investigate the pairwise associations between these partitioned PRSs and relevant clinical endophenotypes from the Danish national health registers. This approach aims to identify functional signatures of gene networks that could point toward novel schizophrenia-related biological mechanisms.

Results: The PRS for schizophrenia, partitioned to gene networks characterized by Neural Progenitor Cell expression, were significantly associated with an ADHD diagnosis in individuals with schizophrenia spectrum disorders, but not in those without. This suggests the potential functional relevance of these gene networks in specific clinical manifestations.

Conclusion: Partitioned PRS provides a promising framework for studying pathways involved in polygenic disorders such as schizophrenia. This approach may, in time, help provide insight into the identification of actionable biological mechanisms of schizophrenia.

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P11.073.D An investigation of the role of tubulin genes in human handedness

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Background: Handedness, the preference to use one hand for specific tasks, is partly influenced by genetics with heritability consistently estimated to be ~25%. A higher prevalence of left-handedness has been reported for different neurodevelopmental traits including language-related conditions. Recent GWAS and whole-exome sequencing studies (WES) suggested tubulin genes are associated with left-handedness. These has been conducted in large population samples e.g. UK Biobank. Here we conducted a target study to assess whether the association between tubulin genes and left-handedness could be detected in smaller samples enriched with language-related difficulties.

Material and Methods: WES data were analysed in a sample of 242 twin children aged 6–11, recruited for an over-representation of developmental language disorders (Wilson and Bishop 2018). Handedness was assessed with the Edinburgh Handedness Inventory. Variants in *TUBB*, *TUBB1*, *TUBB2A*, *TUBB2B*, *TUBB3*, *TUBB4A*, *TUBB4B*, *TUBB6*, *TUBB8*, *ANKS1B*, *MAP2*, *NDRG1*, *NME7*, *TRIM36*, *TUBA1B*, *PTEN*, *LRRK2*, *ZNF207*, *TUBB4BP1*, *TUBB4BP2*, *TUBB4BP3*, *TUBB4BP4*, *TUBB4BP5*, *TUBB4BP6*, *TUBB4BP7* and *TUBB4BP8* were tested for association with left-handedness. Bonferroni correction based on number of tested genes was applied to correct for multiple testing.

Results: We did not observe any significant associations between the tubulin genes and left-handedness, neither did we identify the specific left-handedness associated variants reported by Cuellar-Partida et al (2021) and Schijven et al (2024).

Conclusion: Our study does not provide evidence that variants in tubulin genes influence left-handedness. Our sample size was small and it is possible that tubulin genes influence left-handedness in few individuals requiring large samples for their potential effect to be detected.

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P11.075.B Novel BRAT1 deep intronic variant affects splicing regulatory elements causing cerebellar hypoplasia syndrome: genotypic and phenotypic expansion

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Background: Biallelic mutations in *BRAT1* are implicated in a spectrum of neurodevelopmental disorders, ranging from lethal neonatal rigidity and multifocal seizure syndrome to a milder form characterized by cerebellar atrophy, with or without seizures (NEDCAS). This study investigated three siblings from a consanguineous Bedouin family with NEDCAS, whose causative mutation had eluded standard genetic testing methods.

Material and Methods: Whole-genome sequencing (WGS) was employed to investigate non-coding regions, complemented by linkage analysis using Homozygosity-Mapper. In silico splicing predictions were performed using tools such as SpliceAI, Human Splicing Finder, and ESEfinder. Molecular verification was carried out through Sanger sequencing and real-time PCR to confirm the functional impact of the identified variant.

Results: Using a combination of linkage analysis and WGS, we identified a novel deep intronic *BRAT1* variant (NM_152743.4:c.128-1585T>G) in three siblings presenting with NEDCAS. The siblings exhibited shared clinical features, including cerebellar atrophy, developmental delay, and variable ataxia, with significant phenotypic variability despite identical genotypes. In silico and molecular splicing analyses demonstrated that this variant disrupts splicing regulatory elements, creating a cryptic exon that results in a deleterious frameshift and an aberrant transcript.

Conclusion: This study reports the first deep intronic pathogenic variant in *BRAT1*, highlighting the critical role of intronic splice regulatory elements in disease pathogenesis. It underscores the utility of WGS in resolving unexplained cases and broadens the genotypic and phenotypic landscape of *BRAT1*-related disorders.

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P11.076.C Targeted Next-Generation-Sequencing panels in the diagnosis of hereditary motor neuropathies

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Background: Distal hereditary motor neuropathies (dHMN) are a group of rare heterogeneous disorders characterized by a slowly progressive distal pure motor neuropathy, presenting phenotypic and genotypic overlap with Charcot-Marie-Tooth disease (CMT), amyotrophic lateral sclerosis (ALS), and hereditary spastic paraplegia (HSP). Although <30 genes are associated with these forms, about 60% of cases remain genetically unsolved.

Material and Methods: We analysed a consecutive series of 20 index cases (8 dHMN and 12 predominantly motor CMT) referred to our centre over a period of one year and tested negative for single-gene analysis. NGS analysis was performed by a custom panel including 209 neuropathy-associated genes.

Results: The 65% (13/20) of index cases carried likely pathogenic or pathogenic variants in 9 genes associated to dHMN and predominantly motor CMT. In particular, we identified disease-causing variants in 5 genes associated with autosomal dominant forms, *BICD2* (n = 2), *HSPB1* (n = 1), *GARS1* (n = 1), *DYNC1H1* (n = 1), and *TRPV4* (n = 1), and in 3 genes with autosomal recessive pattern of inheritance, *VRK1* (n = 1), *SORD* (n = 4), *DNAJB2* (n = 1). Moreover, we identified a novel homozygous splicing pathogenic variant in the *RTN2* gene, previously associated with dominant HSP and very recently associated to recessive dHMN.

Conclusion: The use of NGS multigene panel has allowed the genetic diagnosis in 65% of the analysed cases. Compared with previously reported patient series, our results show a higher diagnostic rate confirming the reliability of this approach for the detection of the molecular causes of neuropathies with high genetic heterogeneity.

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P11.077.D Further evidence of *RNU4ATAC* variants causing Joubert syndrome with skeletal involvement

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Background: Joubert syndrome (JS) is a neurodevelopmental ciliopathy defined by the "molar tooth sign" (MTS) on brain imaging and characterized by multiorgan involvement. A recent study has suggested a potential link between JS and biallelic variants in *RNU4ATAC*, a non-coding gene crucial for the correct functioning of the minor spliceosome. While *RNU4ATAC* is already associated with a spectrum of syndromic skeletal dysplasias, its role in JS remains underexplored.

Material and Methods: We reanalyzed whole exome sequencing (WES) data from 53 patients affected by JS, lacking pathogenic variants in known JS-associated genes. The analysis focused on the *RNU4ATAC* locus, which had initially been misannotated as an intronic region of *CLASP1*. Sanger sequencing was performed to confirm and segregate the variants. Clinical and radiological findings were collected and compared to previously described *RNU4ATAC*-related phenotypes.

Results: Compound heterozygous *RNU4ATAC* variants were identified in 3 probands (yield: 6%), including n.16G>A, n.51G>A, n.13C>T, and n.30G>A. All patients displayed the MTS, along with features overlapping JS and other *RNU4ATAC*-related disorders, such as severe intellectual disability, growth retardation, microcephaly, and abnormalities of the skeleton, skin, and genitals.

Conclusion: Our findings support the association between *RNU4ATAC* variants and JS, expanding the phenotypic spectrum of this gene to include this complex neurological-skeletal ciliopathy. The study highlights the importance of accurate WES reanalysis as a key diagnostic tool for uncovering non-coding variants that might otherwise remain undetected. Further research is required to refine genotype-phenotype correlations, explore the frequency of *RNU4ATAC* variants in JS, and assess their long-term clinical impact.

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P11.078.A SNORD3C, a blood biomarker associated to suicide attempts in patients with anorexia nervosa

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Background: Suicide is the second leading cause of mortality in individuals with anorexia nervosa (AN), a psychiatric disorder with high heritability. Despite advances in genomics, the molecular pathways linking AN to suicidality remain poorly understood. This study investigates the role of SNORD3C and related genetic pathways as potential biomarkers for suicide risk in patients with AN.

Material and Methods: Peripheral blood mononuclear cells were collected from a discovery cohort of 15 patients with AN (5 with a history of suicide attempts and 10 without). RNA-sequencing was performed, followed by differential gene expression analysis, pathway enrichment analysis, and replication in an independent cohort of 34 patients. Functional annotations were conducted using STRING and Panther databases to identify dysregulated pathways.

Results: We identified 58 significantly dysregulated genes in the discovery cohort, including SNORD3C, consistently upregulated across cohorts. SNORD3C is a small nucleolar RNA involved in RNA metabolism, priorly associated with psychiatric conditions like depression and vitamin D deficiency. Notably, patients with suicidal behavior indeed exhibited lower vitamin D levels. Pathway analyses revealed enrichment in RNA metabolism and immune pathways.

Conclusion: Our study identifies SNORD3C as a candidate biomarker for suicide risk in AN and confirm the role of immunity in suicidality. The findings provide novel insights into the interplay between genetic and environmental factors in AN and suicidal behavior. Such evidence might lead to the implementation of simple personalized interventions such as vitamine D level management to prevent suicide comorbidity in AN.

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P11.079.B Proteomic insights into SCAR10: Engineering and analysis of CRISPR-edited ANO10 mutant cell lines

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Background: Mutations in the *ANO10* gene cause SCAR10, a rare form of autosomal recessive spinocerebellar ataxia. Anoctamin 10 (ANO10/TMEM16K) functions as both an ion channel and a phospholipid scramblase; however, its exact role in SCAR10 pathogenesis remains unknown. To explore the function of ANO10 regarding SCAR10, four mutant cell lines were generated using CRISPR-Cas9 technology: a knockout and three cell lines carrying known SCAR10-causing variants; c.289delA[p.Thr96_Met97ins*], c.1529T>G[p.Leu510Arg], and c.1009T>G[p.Phe337Val]. Proteomic analysis was performed to identify differentially expressed proteins (DEPs) between mutant and control cell lines.

Methods: SH-SY5Y cells were modified via CRISPR-Cas9 editing, with donor DNA templates facilitating homology-directed repair. Following nucleofection, editing efficiency was evaluated, and single-cell clones were isolated using FACS. Successfully edited clones were expanded and assessed through immunoblot for ANO10 expression. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was employed to identify differentially expressed proteins between control and mutant cell lines after differentiation. DEPs were identified based on a fold-change of ≥ 2 or ≤ 0.5.

Results: Four ANO10-modified cell lines were successfully engineered and established. Western blot analysis confirmed ANO10 downregulation, and the edited cell lines showed no off-target effects. Proteomic analysis showed that 473, 717, 757, and 1142 proteins were significantly differentially expressed in SH-SY5Y ANO10$^{-/-}$, c.289delA, c.1529T-G, and c.1009T-G cell lines, respectively, compared to wild-type cells.

Conclusion: The engineering and analysis of ANO10-modified cell lines revealed significant proteomic changes associated with SCAR10 mutations. Further investigation is expected to uncover the biological pathways involved in SCAR10 pathogenesis and provide deeper insights into the disease biology.

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P11.080.C Identification of rare variants for Anorexia Nervosa in Genomics England database

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Background: Anorexia nervosa is a severe eating disorder characterized by restrictive eating and low body weight, with the highest mortality rate among psychiatric disorders. Given its high heritability (50–60%), identifying key genetic contributors is crucial for developing effective treatments.

Material and Methods: As part of the Genomics England 100K project, we recruited a cohort of individuals with severe familial anorexia nervosa and their relatives (57 cases, 52 relatives), who underwent whole-genome sequencing using Illumina short-read technology. Additional cases ($n = 97$) were recruited for other studies across the broader 100K program. Our primary objective was to investigate rare genetic variants contributing to anorexia nervosa risk. To limit the search space, we used data from a 2019 GWAS of AN, which previously identified eight genome-wide risk loci, including 4 single loci: *CADM1*, *MGMT*, *FOXP1*, and *PTBP2*. Using summary statistics from this GWAS, we applied KGWAS, a deep-learning tool leveraging a functional knowledge graph of variants and genes to identify reliable associations.

Results: The KGWAS analysis confirmed *FOXP1* and *CADM1* as significant single-gene loci. Within the Genomics England Research Environment, we used bcftools and the Interactive Variant Analyser to assess whether anorexia nervosa participants ($n = 154$) harboured damaging variants in these two loci at an allele frequency of <0.01. Our preliminary analyses revealed 9 participants with damaging variants in *FOXP1* and 16 participants with damaging variants in *CADM1*.

Conclusion: Rare damaging variants in *FOXP1* and *CADM1* further implicate these genes as important factors in the aetiology of AN.

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P11.081.D Deciphering the heterogeneous pathomechanisms of ASD/ID Using a statistically validated clinical score

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Background: The nosological classification of neurodevelopmental disorders (NDDs), particularly autism spectrum disorder (ASD), remains ambiguous. NDDs exhibit a complex etiology that may be monogenic or multifactorial. While genomic advances have facilitated the identification of causative genes, the diagnostic yield of exome sequencing (ES) is significantly higher in monogenic cases than in multifactorial ones.

Aim: (1) Refine the nosological classification of NDDs in relation to pathogenesis, (2) define the appropriateness of genetic testing, and (3) establish a standardized diagnostic workflow.

Methods: We analyzed standardized clinical data from 500 consecutive patients who underwent genetic counseling at Policlinico Gemelli for the diagnosis of NDDs, all of whom underwent pre-test genetic evaluation. By comparing clinical assessments with genetic findings, we identified 14 predictive criteria distinguishing monogenic from multifactorial ASD/intellectual disability (ID).

Results: Statistically significant differences in clinical phenotypes allowed us to develop a clinical scoring system incorporating prenatal anomalies, birth abnormalities, hypertonia/hypotonia, delayed motor milestones, epilepsy/EEG abnormalities, dysautonomia, respiratory abnormalities, moderate/severe ID, concurrent ASD and ID, congenital heart defects, CNS anomalies, macrocephaly/microcephaly, dysmorphic features, and a non-Mendelian family history of neuropsychiatric disorders. Most multifactorial cases had a score of ≤3, while most monogenic cases had a score of ≥4.

Conclusions: We propose guidelines for genetic testing in ID/ASD to optimize its application, limit testing in primary ASD, and facilitate the selection of families with primary ASD for omics studies.

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P11.082.A NBIA diagnosis : lessons from ten years experience in a French reference lab

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Background: Neurodegeneration with Brain Iron Accumulation (NBIA) comprises a group of rare, inherited disorders characterized by abnormal iron deposition in the basal ganglia, detectable via specific brain MRI sequences. NBIA is genetically and clinically heterogeneous, with variable presentations and ages of onset.

Material and Methods: We analyzed 363 patients with clinical and radiological features suggestive of NBIA. Targeted Sanger sequencing of *PANK2* and *FTL* or a next-generation sequencing (NGS) panel of 9 genes (*ATP13A2, CP, DCAF17, FA2H, FTL, C19orf12, PANK2, PLA2G6, WDR45*) was performed. Whole-genome sequencing was conducted in cases with negative panel results but strong clinical suspicion of NBIA.

Results: A molecular diagnosis was achieved in 78 patients, with *PANK2* being the most frequently implicated gene. Detailed analyses of age at onset, initial symptoms, and main clinical manifestations were performed for each subtype.

Conclusion: Our study identified atypical NBIA forms, including four families with dominant MPAN due to *C19ORF12*variants, two of which involved mosaic carriers with late-onset presentations, the only reported mosaic cases for *C19ORF12* to date. Additionally, post-zygotic mosaicism was suspected in monozygotic twins, clinically discordant for a pathogenic *WDR45* variant. We also described an attenuated form of Woodhouse-Sakati syndrome in a compound heterozygous patient (*DCAF17*), carrying both a missense and a nonsense variant, with missense variant never described in *DCAF17*.

Our work provides detailed genetic, clinical, and radiological descriptions of NBIA, highlights atypical phenotypes, and underscores the utility of trio exome and genome sequencing in identifying novel genetic causes of iron accumulation.

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P11.083.B Biallelic *RFC1* expansions as a rare cause of familial and early onset Parkinson's disease in the Slavic population

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Background: Parkinson's disease is a common, progressive neurodegenerative disease, presenting with tremor, bradykinesia, muscle rigidity, and non-motor symptoms. Only 10% early-onset and familial Parkinson's disease patients (EOPD/FPD) receive a genetic diagnosis. The cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is a rare type of late-onset ataxia primarily caused by biallelic pathogenic $(AAGGG)_n$ repeat expansions in the *RFC1* gene. While recent evidence shows genetic overlap between EOPD and CANVAS, but its prevalence in FPD remains unknown. Our aim was to determine the possible presence of CANVAS-specific pathogenic expansions in a well-characterized cohort of 135 FPD and EOPD patients with previously negative genetic testing.

Material and Methods: We performed CANVAS testing by using a two-step fluorescent polymerase chain reaction (PCR), based on the initial exclusion of long expansions in CANVAS, and a second stage confirmation of long pathogenic expansions, with the detection based on fragment analysis. Finally, we tested for SNV pathogenic variants in RFC1 gene using reinterpretation of exome sequencing data.

Results: By applying this method, we found two patients with homozygous pathogenic expansions in *RFC1* in our cohort of FPD and EOPD patients. We show biallelic pathogenic CANVAS variants are present in 1.5% of our independent FPD and EOPD cohort,

which in our population makes them more common than the established autosomal recessive PD genes such as PARK2.

Conclusion: Based on these results, we suggest biallelic *RFC1* pathogenic expansions should be considered when performing genetic diagnostics of FPD and EOPD.

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P11.084.C Combined family history and polygenic score prediction of anxiety disorders

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Background: Anxiety disorder is a complex psychiatric disorder influenced by genetic, social, and environmental factors. This study modelled the combined effects of family history of multiple psychiatric disorders (mFH), polygenic risk scores of multiple traits (mPRS), childhood trauma (ChT) and sociodemographic factors on the development, recurrence, and duration of generalized anxiety disorder (GAD) and panic disorder (PD).

Material and Methods: Data were obtained from the Genetic Links to Anxiety and Depression Study (GLAD+) and UK Biobank (UKB). Diagnosis of GAD and PD were based on DSM-5 criteria. Family history was reported for 9 psychiatric disorders. MegaPRS was used to calculate PRSs based on large genome-wide association studies. Childhood trauma was assessed via the five-item childhood trauma screener. Elastic net regression with nested cross-validation was applied to select the most associated predictors.

Results: In GLAD+, mFH, mPRS, ChT, and sociodemographic factors explained 40% of GAD variance (5,128 cases and 4,226 controls, AUC-ROC = 0.86) and 38% of PD variance (3,470 cases and 4,459 controls, AUC-ROC = 0.86). These predictors accounted for 28% and 22% of variance in the number of episodes for GAD and PD, and 32% of variance in episode duration for both conditions. In UKB, these predictors explained 24% of GAD variance (19,746 cases and 66,233 controls, AUC-ROC = 0.76) and 36% of PD variance (5,057 cases and 28,298 controls, AUC-ROC = 0.82).

Conclusion: Integrating mFH, mPRS, ChT, and sociodemographic factors provides a robust model for predicting GAD and PD risk, recurrence, and chronicity. This approach may support early diagnosis and personalized interventions for anxiety disorders.

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P11.085.D Genetic epidemiology of congenital and very-early-onset ataxia

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Between 2015 and 2024, 810 individuals with congenital ataxia were referred to our reference center on Cerebellar Malformations and congenital diseases in order to identify the genetic etiology. All patients presented clinical cerebellar symptoms before the age of two years. We excluded i) patients with a clearly progressive ataxia at the time of inclusion, ii) patients whose brain MRI were suggestive of a specific diagnosis (Joubert syndrome, acquired cerebellar damage, etc), and iii) patients with cerebellar atrophy without clinical ataxia (intellectual disability, severe encephalopathy). We carried out a systematic genetic analysis that has evolved over the years, from Sanger sequencing to high-throughput sequencing. Three successive NGS genes panels allowed a total diagnostic yield of 35%. Pangenomic analysis (WES or WGS) is ongoing in patients without a genetic diagnosis after the first screening. We will present the genetic epidemiology in congenital ataxia in our cohort, and genotype-phenotype analysis. As previously reported, pathogenic variants in ITPR1 and CACNA1A, were among the most frequent diagnoses. We were able to identify several patients affected with a metabolic disease with a very insidious onset mimicking a congenital ataxia. WES/WGS has already increased the diagnosis rate to 46 % and allowed identification of new genes. However, regarding the high diagnostic yield of the panel, WES was not used at first-line. As validation of the variants generated by NGS is now the main challenge, we are developing RNAseq analysis routinely in our laboratory.

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P11.086.A Gut microbiome changes in major depressive disorder and investigation of causality by Mendelian Randomization and mediation

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Background: The gut microbiome composition, which is a potential predictor of the host's health status, is determined by diet, medication, and host genetics. The current study explored the intricate links between the gut microbiome and major depressive disorder (MDD) in the Atlas Biomed discovery and replication studies.

Material and Methods: The microbiome was characterised by 16S rRNA gene sequences, QCed and processed by QIIME2 to get

the relative abundance of each bacterial genus. Linear regression was used to assess the association between MDD and bacterial relative abundance in both discovery (544/3,912 cases/controls) and replication (285/2,167 cases/controls), adjusting for region, age, gender, and batch effects. Meta-analysis of each association summary statistics was performed using a random effects model. Mendelian Randomization (MR) and mediation analysis were used to assess causal links.

Results: Meta-analysis identified nine taxa significantly associated with MDD, including Romboutsia, Clostridium sensu stricto, Agathobacter, Bacteroides pectinophilus group, Flavonifractor, Coprococcus, Victivallis, Tyzzerella, and Bacteroides ($q < 0.05$). MR analysis did not support causal relationships between MDD and bacterial abundances of associated taxa. Mediation analyses showed that Bacteroides mediated 61% of total lifestyle effects on MDD, with notable contributions of healthy weight, physical activity, and diet. Integrating multiple bacterial taxa into a microbiome risk score outperformed single-taxon associations, achieving an AUC of 0.6 and demonstrating its potential to capture broader microbial patterns.

Conclusion: Our findings reveal consistent microbial signatures linked to MDD and underline the importance of the microbiome in studying psychiatric outcomes.

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P11.087.B *AP4B1* hypomorphic variants cause autosomal recessive adult-onset ataxia

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Background: Biallelic loss-of-function variants in *AP4B1* have been primarily documented in hereditary spastic paraplegia type 47 (SPG47, MIM 614066), a severe genetic disorder characterized by a broad spectrum of symptoms encompassing global developmental delay, intellectual disability and infantile-onset hypotonia evolving into spastic diplegia with progressive loss of independent ambulation. Although late-onset cerebellar ataxia, along with other related neurodegenerative diseases, has recently been associated with high rate of hypomorphic variants in various severe disease-causing genes, involvement of AP4B1 in such moderate phenotypes has never been established thus far.

Methods: Through a national collaboration, clinical and molecular data of three previously unreported individuals displaying a sporadic form of isolated adult-onset ataxia associated with compound heterozygous variants in *AP4B1* were systematically gathered, and a comparative analysis was conducted between this series and existing literature.

Results: Affected individuals we are documenting manifest comparable forms of slowly evolving adult-onset ataxia, primarily characterized by gait ataxia coupled with progressive dysarthria and moderate cerebellar atrophy. Pangenomic analyses identified two novel missense variants, namely c.320G>A p.(Arg107Gln) and c.1558C>T p.(Arg520Cys), detected in *trans* with either a frameshift or a canonical splicing site variant in *AP4B1*.

Discussion: These pathogenic missense variants are strongly suspected to act as hypomorphic, thereby allowing for residual AP4B1 activity consistent with the attenuated phenotype observed across this series. From a broader standpoint, this study will contribute to refine the genetic landscape currently involved in sporadic late-onset ataxia, while assisting molecular geneticists in the interpretation of pangenomic analyses conducted on individuals displaying mild neurological phenotypes.

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P11.088.C Trio-WES in pediatric-onset epilepsy: assessing diagnostic yield and widening genotypic landscape

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Background: Whole-Exome Sequencing (WES) is a cost-effective genetic test for epilepsy with an expected diagnostic yield of ~32%. We aim to assess the diagnostic yield of trio-based WES in pediatric-onset epilepsy, identify novel variants associated with epilepsy, and evaluate the clinical impact of genetic diagnosis.

Material and Methods: 30 patients with pediatric-onset epilepsy were enrolled in EPIEXOME, a prospective monocentric study on genetic epilepsies, and underwent trio-WES. The variants were classified according to ACMG criteria.

Results: The cohort included 11 patients with isolated epilepsy and 19 with cognitive and/or motor disturbances. Epilepsy was focal in 15/30 and generalized in 15/30 cases. Mean age at onset was 5.75 years [0-16]. Trio-WES resulted positive in 11/30 (37%, table 1), uncertain in 8/30, and negative in 11/30 cases. Among positive cases, 7 variants were de novo, one variant was inherited in autosomal dominant fashion by an affected father, 3 cases were recessive.

Conclusion: In a monocentric cohort, trio-WES achieved a diagnostic result in 37% of the cases. The high representation of de novo variants stresses the relevance of trio analysis in epilepsy. We link *GABRB1* gene with two novel epileptic phenotypes (EIMFS and Lennox-Gastaut Syndrome) and report a case of steroid-responsive para-infectious acute encephalopathy in a patient with biallelic *QARS1*-variants. The clinical impact of genetic diagnosis included personalized therapeutic possibilities (*KCNT1*, *CACNA1A*, *QARS1*), reverse phenotyping with systemic evaluation (*SON*), and adhesion to patient advocacy groups (*SLC6A1*).

Table 1:

ID	Gene	Transcript	Variants	Status	ACMG
1	*SLC6A1*	ENST00000287766.4	c.1070C>T p.Ala357Val	Heterozygous (de novo)	P
5	*GABRB1*	ENST00000295454.3	c.841A>G p.Thr281Ala	Heterozygous (de novo)	LP

ID	Gene	Transcript	Variants	Status	ACMG
9	*TUBA1A*	ENST00000301071.7	c.97G>A p.Asp33Asn	Heterozygous (de novo, mosaicism)	LP
13	*KCNT1*	ENST00000298480.5	c.2800G>A p.Ala934Thr	Heterozygous (de novo)	P
15	*SON*	ENST00000290239.6	c.6064dup p.Thr2022AsnfsTer8	Heterozygous (de novo)	P
19	*NEXMIF*	ENST00000055682.6	c.1441C>T p.Arg481*	Heterozygous (de novo)	P
20	*CACNA1A*	ENST00000360228.5	c.3946G>A p.Asp1316Asn	Heterozygous (affected father)	LP
22	*QARS1*	ENST00000306125.6	c.799C>T p.Arg267Trp c.1304A>G p.Tyr435Cys	Compound heterozygous	VUS LP
24	*KMT2E*	ENST00000311117.3	c.1725_1726delAA p.Thr575 = fs	Heterozygous (de novo)	P
29	*KCNJ10*	ENST00000368089.3	c.557T>C p.Val186Ala c.886G>A p.Val296Met	Compound heterozygous (likely)	LP LP
30	*PEX7*	ENST00000318471.5	c.120C>G p.Tyr40* c.347G>T p.Ser116Ile	Compound heterozygous	P LP

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P11.089.D Unraveling the genetic landscape of ALS in Greece: identification of known and novel causative variants in a 353-patient cohort

Chrisoula Kartanou[1], *Zoi Kontogeorgiou*[1], *Theodoros Loupis*[2], *Dimitrios M. Vrachnos*[2], *Nikolaos Ragazos, MD*[1], *Ifigenia Spyropoulou*[1], *Maria Petraki*[1], *Chrysoula Koniari*[1], *Stavroula Aristeidou*[3], *Eleftheria Koropouli*[3], *Ariadne Daponte*[3], *Michail Rentzos*[3], *Elisabeth Kapaki*[3], *Marios Panas*[1], *Periklis Makrythanasis*[4,5,6], *Leonidas Stefanis*[3], *Georgios Koutsis*[1], *Georgia Karadima*[1]

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Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive, fatal neurodegenerative disorder characterized by progressive loss of motor neurons. Approximately 15% of individuals diagnosed with ALS have a known genetic variant that contributes to disease. Herein, we present clinical and genetic data of a large Greek ALS cohort.

Patients and Methods: The cohort consisted of 353 Greek consecutive index patients with ALS. Next generation sequencing raw data (obtained from the NYGC ALS Consortium) were further analyzed and used to screen for causative variants in known implicated genes. Repeat expansions in *C9ORF72* and *ATXN2* were investigated using ExpansionHunter software, repeat-primed PCR and fragment analysis.

Results: Pathogenic repeat expansions in *C9ORF72* were detected in 41 patients (11.6%). In addition, 30 patients (8.5%) carried a causative variant in one of the genes studied. Known causative variants were identified in 27 cases (9 in *SQSTM1*, 7 in *TARDBP*, 5 in *SOD1*, 3 in *NEK1* and 1 each in *SETX*, *VCP*, *FUS*), whereas novel causative variants were identified in 3 cases (*SOD1*, *FIG4*, *TBK1*). In total, 71 cases received a molecular genetic diagnosis (20.1%). Additionally, 7 cases (2.0%) carried an intermediate repeat expansion (30–33 CAG) in *ATXN2*.

Conclusion: Our results reveal the distinct genetic profile of Greek ALS patients. These findings will have an impact on genetic counselling, the design of diagnostic gene panels for the Greek population and on genotype-specific therapeutic interventions. Understanding the genetic causes of ALS in different populations is becoming increasingly important, especially with the advent of personalized medicine.

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P11.090.A Alpha-synucleinophaties in Gaucher disease: a multicenter longitudinal study

Marco Percetti[1], *Edoardo Monfrini*[1], *Emanuele Frattini*[1], *Giulia Franco*[1], *Ilaria Trezzi*[1], *Maria Chiara Malaguti*[2], *Francesco Cavallieri*[3], *Franco Valzania*[3], *Giovanni Cossu*[4], *Silvia Ramat*[5], *Alessandra Govoni*[5], *Cristoforo Comi*[6], *Alessandro Tessitore*[7], *Rosa De Micco*[7], *Antonio Barbato*[8], *Paolo Tirelli*[9], *Francesca Spagnolo*[10], *Maria Domenica Cappellini*[1], *Elena Cassinerio*[1], *Irene Motta*[1], *Natalia Scaramellini*[1], *Francesca Carubbi*[11], *Fabio Nascimbeni*[12], *Lorenza Borin*[13], *Lorenza Borin*[13], *Emanuele Cerulli*[14], *Fiorina Giona*[14], *Alessio Barnaba Di Fonzo*[1]

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Background: Gaucher disease (GD) is a lysosomal storage disorder caused by biallelic *GBA1* variants, which represent the most common genetic risk factor for Parkinson's disease (PD) and dementia with Lewy bodies (DLB). The aim of the study was to investigate the occurrence of PD and DLB in a cohort of type 1 and type 3 GD patients (GD1, GD3), and to identify possible predictors of PD or DLB phenoconversion in these patients.

Material and Methods: GD patients from multiple centers were consecutively evaluated by neurologists with expertise in movement disorders at two different time-points (T0, at baseline; T1, after 4 ± 2y).

Results: One-hundred-thirty-six GD1 and 13 GD3 patients were evaluated at T0 (mean age-at-T0 47.6 ± 15.6y). The mild N409S variant in compound heterozygosity with another *GBA1* variant (N409S/other) was the most frequent genotype (72.1%). Diagnosis of PD and DLB was reached in 18 (13.2%) and two (1.5%) GD1 patients, respectively. Fifty-four GD1 and five GD3 patients were re-evaluated at T1. PD and DLB phenoconversion were observed in four (7.4%) and seven (13%) GD1 patients, respectively. Two

(15.4%) GD3 patients developed PD. PD diagnosis preceded that of GD in six (4.4%) GD1 patients. The lifetime risk to develop PD was 25.5% in N409S/other GD1 patients and 0% in homozygous N409S patients at 60y ($p = 0.003$), 34.8% and 33.3% respectively at 70y ($p = 0.54$).

Conclusion: This study highlights the potential of close follow-up of GD patients to identify possible predictors of phenoconversion to PD or DLB.

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P11.091.B Genomic SEM and GWAS subtraction: isolating mania's genetic architecture in bipolar disorder

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Background: Bipolar disorder is characterized by episodes of mania and depression, often accompanied by psychotic symptoms. While substantial progress has been made in unraveling the genetic basis of depression and psychosis, the genetic underpinnings of mania remain relatively understudied.

Material and Methods: We applied Genomic Structural Equation Modeling to disentangle the genetic contributions of major depressive disorder (MDD) from bipolar disorder, aiming to isolate a genetic factor specific to mania.

Results: The SEM model demonstrated strong factor loadings for "mania" (0.90, $p < 0.001$) and "depression" (0.43, $p < 0.001$), with mania and MDD accounting for 81.5% and 18.5% of the genetic variance in bipolar disorder, respectively. Seventy-one genome-wide significant SNPs linked to mania were identified across 37 genomic loci. Furthermore, mania displayed distinct genetic correlations compared to bipolar disorder in relation to psychiatric, substance use, somatic, social, and neurological traits. Notable differences were observed in genetic correlations with tiredness ($rg = -0.13$ vs -0.17), subjective well-being ($rg = 0.08$ vs -0.13), and educational attainment ($rg = 0.26$ vs 0.14). Pathway analysis revealed significant enrichment in the voltage-gated calcium channel activity pathway (GO:0086007).

Conclusion: These results refine our understanding of the genetic architecture of bipolar disorder, potentially enabling a more mania-specific GWAS. This knowledge could inform future therapeutic approaches, particularly targeting calcium channel pathways in the treatment of mania.

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P11.092.C Genetic Prevalence of Cerebral Cavernous Malformations in the UK Biobank

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Background: Cerebral cavernous malformations (CCM) consist of clusters of irregular dilated capillaries and represent the second most common type of vascular malformation affecting the central nervous system. CCM might be asymptomatic or cause cerebral hemorrhage, seizures, recurrent headaches and focal neurologic deficits. Causative mutations underlining CCM have been reported in three genes: KRIT1/CCM1, MGC4607/CCM2 and PDCD10/CCM3. Therapeutic avenues are limited to surgery. This condition is likely underestimated: up to 50% remain asymptomatic for life. We aimed to determine the prevalence of CCM disease in UK Biobank.

Material and Methods: We sought KRIT1, CCM2 and PDCD10 gene variants in exome sequencing data from 470 000 individuals aged 40 years or older). We focused on variants previously established as pathogenetic as well as novel nonsense variants or variants impacting (in silico validation) on canonical splice sites. We compared demographic data and ICD-10 (International Classification of Diseases version-10) codes with those without pathogenic variants.

Results: We identified 68 different pathogenetic molecular defects (KRIT1: 41; CCM2: 26; PDCD10: 1). 172 subjects (KRIT1 73%; CCM2 26%, PDCD10 <1%) carried one of these pathogenetic alleles. Male to female ratio was equal to 1. 88% of the mutated subjects were asymptomatic at enrollment.

Conclusion: The genetic prevalence of CCM disease in UK Biobank (1:2700) exceeds the known estimate of the genetic form (1:5000 - 1:10000). Incomplete and age-dependent penetrance likely explains the reduced number of symptomatic patients. Clinical and radiological monitoring is fundamental to prevent neurological complications of CCM.

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P11.093.D WES analysis in individuals with dyslexia and reading difficulties

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Background: Dyslexia is a specific difficulty in learning to read, significantly influenced by genetic factors. Recently, we reported whole exome sequencing (WES) in 53 cases with dyslexia (https://doi.org/10.1101/2024.12.19.24319320). The top results were followed up in a replication sample of 38 individuals with reading difficulties, highlighting five genes of interest, including two voltage-gated calcium channel genes.

Methods: We reanalysed our data by combining the two WES datasets (N = 53 + 38) and an additional 17 individuals with dyslexia (Ntotal = 108). We applied filtering criteria based on allele frequency (MAF in gnomAD < 1%) and high predicted pathogenicity. FUMA was utilised for gene-set enrichment analysis (GSEA). An association analysis of the top variants with cognitive and psychiatric traits was conducted in the UK Biobank.

Results: The *CFTR* gene had the highest number of variants, with 10 distinct variants identified among 12 individuals. Additionally, rare coding variants in *ABCA4*, *CLDN3*, and *FLNC* were each observed in six individuals. In a phenotyped control group (N = 82), we observed two variants in *CFTR* and one in *FLNC*. One variant was observed in *CLDN3* but differed from the single variant detected in the cases. GSEA highlighted pathways related to ion transport and the activity of voltage-gated channels. A UK Biobank preliminary analysis found no associations.

Conclusion: Our WES supports the role of rare variants in dyslexia. In line with patterns observed in other traits, the lack of association in general population samples suggests that the impact of rare variants may be observable in clinical samples that could be enriched for additional risk factors.

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P11.094.A Diagnostic yield and copy number variants findings in 220 adult patients with developmental and epileptic encephalopathies

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Background: Most Developmental and epileptic encephalopathies (DEE) are caused by pathogenic de novo SNV in genes, each contributing to a limited proportion of cases[1]. WES is the most convenient approach[2], although chromosomal microarray for CNVs detection would be the first-tier genetic test for patients with DEE and/or dysmorphisms[3]. Most of the studies on CNVs focused on paediatric cohorts[4], data on adults are scant. We investigate the role of CNVs in a cohort of adult DEE.

Material and Methods: Patients with undiagnosed DEE underwent CNV analysis by Agilent Technologies array-CGH 8 × 60K platform and Cytogenomics software or SNP-array from Epi25 data using PennCNV and AnnotSV software for variant calling and annotation.

Results: 220 unrelated DEE patients (M/F:104/116, mean age at seizures onset:9.3 ± 5.04 years) underwent array-CGH or SNP-array at a mean age of 35.13 ± 14.95 years. We identified causative CNVs in 18 (8.2%), 8 of whom de novo (44.4%): del1p36.33-p36.23, dup1q21.1q21.2, del2p16.3, mosaic del2q23, del2q23.3q24.2, del2q37.3, del3p14.2p14.1, del3q29, del6q27, del13q13.2q13.3, del15q11.2q13.1 compatible with Angelman syndrome, dup15q11.2q13, 15q13.2q13.3 × 3+15q11.2q13.2 × 4 compatible with an inv-dup (15) syndrome, dup16p13.1, del 17q21.31, del 19q12-q13.11, del 22q11.2, del 22q13.33. The mean age at diagnosis was 32.4 ± 13 years. Of the remaining, 24(10.9%) had VUS, 178(80.9%) were negative.

Conclusions: We identified causative CNVs in 8.2% of patients with a diagnostic delay of 23 years. The diagnostic yield aligns with data on pediatric series. Although WES is recommended as the initial approach to reduce diagnostic costs and time, incorporating CNV analysis is recommended in the diagnostic workflow for adult patients with DEE, as well.

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P11.095.B Genetic overlap between dementia and psychiatric disorders: insights from GWAS and genomic SEM

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Background: Epidemiological and clinical studies suggest an association between psychiatric disorders and increased dementia risk (1–3), with evidence indicating that dementia develops earlier in individuals with psychiatric disorders (4). However, the genetic basis of these associations remains unclear.

Material and Methods: We investigated the genetic correlation between dementia subtypes (alzheimer's disease, vascular dementia (VaD), frontotemporal dementia subtypes (FTD, FTDbv, FTDmnd, FTDpnfa, FTDsn), lewy-body dementia) and psychiatric disorders (attention-deficit/hyperactivity disorder (ADHD), problematic alcohol use (ALCH), cannabis use disorder (CUD), autism spectrum disorder (AUT), anxiety disorders (ANX), bipolar disorder subtypes (BD, BD1, BD2), depressive disorder (MDD), post-traumatic stress disorder (PTSD), schizophrenia (SCZ)) using summary statistics from genome-wide association studies. LD Score regression (LDSC) was used to estimate genetic correlations (rG), and genomic SEM was applied to model the genetic covariance structure, revealing a well-fitting five-factor model (Dementia, Affected, Neurodevelopment, Psychotic, Substance).

Results: VaD showed significant genetic correlations with ADHD, CUD, ALCH, BD, ANX, MDD, and SCZ. BD1 was specifically correlated with VaD, while BD2 was linked to FTDbv. The dementia factor was significantly correlated with affected (rG = 0.13, p = 0.01), substance (rG = 0.24, p = 0.008), and psychotic (rG = 0.18, p = 0.001) factors.Table: Significant genetic correlations estimated using LDSC

Dementia subtypes	Psychiatric disorders	rG	SE	Z-score	p-value
FTDbv	BD2	0.6024	0.3026	1.9907	0.0465
VaD	ADHDadult	0.3055	0.1275	2.3961	0.0166
VaD	ADHDchild	0.3091	0.1077	2.8700	0.0041
VaD	CUD	0.3110	0.1239	2.5101	0.0121
VaD	ALCH	0.3012	0.1222	2.4648	0.0137
VaD	BD	0.3394	0.1385	2.4505	0.0143
VaD	BD1	0.2086	0.0918	2.2723	0.0231

Dementia subtypes	Psychiatric disorders	rG	SE	Z-score	p-value
VaD	ANX	0.2744	0.1205	2.2772	0.0228
VaD	MDD	0.2303	0.0731	3.1505	0.0016
VaD	SCZ	0.2014	0.0765	2.6327	0.0085

Conclusion: Our findings highlight a strong genetic association between dementia and psychiatric disorders, with VaD exhibiting the most extensive shared genetic background. Additionally, BD subtypes show distinct genetic correlations with different dementia subtypes. Identifying common biological pathways may aid in dementia prevention and the development of targeted therapies for psychiatric disorders.

C.O.I. Disclosure:

G.S. Can: None. **H. Şeker:** None. **Y. Oktay:** None.

P11.096.C Global DNA methylation level and mRNA expressions of DNMTs in Alzheimer disease peripheral blood cells

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Background: Epigenetic mechanisms, particularly DNA methylation, have emerged as crucial regulators of gene expression in both health and disease contexts. Global DNA methylation levels, along with the activity of DNA methyltransferases (DNMTs), play a pivotal role in maintaining genomic stability and regulating gene transcription. This study aims to evaluate global DNA methylation levels and mRNA expression of DNMT1 and DNMT3b in peripheral blood cells from Alzheimer's disease (AD) patients.

Material and Methods: Relative mRNA levels of DNMT genes, were examined in 69 AD patients and 33 controls by RT-PCR. Global DNA methylation levels were measured using ELISA in 8 AD and 7 controls.

Results: We found an increase in global DNA methylation in AD patients compared to controls ($p = 0.028$). DNA methylation negatively correlated with age (Spearman $r = -0.62$, $p = 0.017$). The expression of DNMT1 and DNMT3b, was decreased in AD patients compared to healthy controls ($p < 0.05$).

Conclusion: This study highlights significant epigenetic alterations in Alzheimer's disease (AD), providing evidence of increased global DNA methylation and reduced mRNA expression of DNMT1 and DNMT3b. The observed inverse correlation between DNA methylation levels and age further underscores the complex interplay between aging and epigenetic regulation in AD pathogenesis. These findings suggest that dysregulated DNA methylation and impaired activity of DNMTs may contribute to the molecular mechanisms underlying AD. Future research should aim to expand sample sizes, investigate the functional consequences of these epigenetic changes, and explore their potential as biomarkers for early diagnosis or therapeutic intervention in AD.

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P11.097.D Whole Exome Sequencing Revealed Ultra-Rare Genetic Variations in Juvenile Myoclonic Epilepsy

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Background: Juvenile Myoclonic Epilepsy (JME) is the most common adolescent and adult-onset genetic generalized epilepsy. In this study, we aimed to identify all rare variants present in exons and exon-intron junctions in patients who met the criteria of JME, determine potentially pathogenic variants, and find the assumed genotype/phenotype correlation between the identified variants and the JME clinical features.

Material and Methods: Whole Exome Sequencing (WES) was performed for ten JME patients from different families. Validation, co-segregation and mode of inheritance were determined using Sanger DNA sequencing.

Results: Predictable damaging variants were found in six families with positive co-segregation. Eight variants in eight genes (SCN1B, KCNQ2, CACNA1I, GABRA3, BSN, RYR3, SEZ6, and RYR2) and one novel variant in (TNR) gene were found to be associated with JME. All these genes play key roles in the interactions between neurons, neurotransmitter release, and maintenance of the balance between neuronal excitation and inhibition.

Conclusion: Since the identified genes are involved in the molecular mechanisms underlying seizures, such variants can potentially be epileptogenic. In conclusion, the identified variants that co-segregate with JME symptoms and likely contribute in creating the adequate genetic background for the JME phenotype.

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P11.098.A Genetic study of copy number variants in Greek patients with early-onset Parkinson's disease

Nikolaos Ragazos[1], *Chrisoula Kartanou*[1], *Zoi Kontogeorgiou*[1], *Nikolaos Papagiannakis*[2], *Christos Koros*[2], *Athina Simitsi*[2], *Ioanna Alefanti*[2], *Matina Maniati*[3], *Georgia Xiromerisiou*[4], *Iro Boura*[5], *Cleanthe Spanaki*[5], *Leonidas Stefanis*[2,3], *Georgios Koutsis*[1], *Georgia Karadima*[1]

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Background: Parkinson's disease (PD) is a multifactorial progressive neurodegenerative disorder characterized by motor and non-motor symptoms. PD is the second most prevalent neurodegenerative disorder. Monogenic forms account for about 10–15% of PD cases and are often characterized by early onset (<50 years). Single nucleotide variants (SNVs), insertion or deletion (Indels) and copy number variants (CNVs) in PD-associated genes have been detected across many populations and ethnic groups. This study aimed to estimate the frequency of CNVs in Greek patients with early-onset PD (EOPD).

Patients and Methods: The study cohort consisted of 71 Greek index EOPD patients. All patients had been previously screened for the pathogenic variants p.A30G and p.A53T in *SNCA* (common causes of PD in the Greek population) by RFLP, and for causative variants in other PD-associated genes by next generation sequencing and tested negative. Multiplex ligation-dependent

probe amplification (MLPA) was used for the molecular investigation of CNVs

Results: MLPA identified a heterozygous deletion in exon 2 of the *PARK2* gene in a single patient known to harbor a pathogenic SNV in heterozygosity in *PARK2*. This was a sporadic case with age at onset of 30 years and mild disease course. The frequency of CNVs in this EOPD cohort was 1.4%.

Conclusion: The detection rate of CNVs in the present study is in accordance with previous studies in other populations. Our findings may contribute to the development of an appropriate diagnostic algorithm for genetic testing of Greek patients with EOPD, providing timely and accurate diagnosis.

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P11.099.B Acute childhood psychosis - ultra-rapid genome sequencing confirms a rare presentation of Radio-Tartaglia syndrome

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Background: Radio-Tartaglia syndrome is a rare neurodevelopmental disorder caused by *SPEN* haploinsufficiency. This is characterized by developmental delay, intellectual disability, multiple congenital anomalies, and facial dysmorphism. Individuals with neurodevelopmental conditions have a higher prevalence of mental health conditions, and early identification of these concerns can improve their quality of life. Children with mental health concerns should be evaluated for features of genetic neurodevelopmental conditions, as genomic testing facilitates accurate diagnosis and targeted interventions. **Case**: We report a case of a 12-year-old boy of Vietnamese background who presented with Cotard delusions. This is a condition rarely reported at this age, where an individual believes they are dead. Additionally, he had periventricular heterotopia on MRI and exhibited bilateral upper limb dystonia and slow upward saccades on examination.

Results: Ultra-rapid trio genome sequencing revealed a de novo heterozygous pathogenic variant in *SPEN*, c.9766_9767insA which is predicted to result in nonsense mediated decay.

Discussion: A literature review has identified 38 individuals with *SPEN* haploinsufficiency, yet our patient presents a unique clinical presentation. In this case, genomic diagnosis informed the therapeutic approach, guiding the decision to utilise a mood stabiliser rather than traditional antipsychotic medications. As genome sequencing is becoming more widely used in clinical medicine, further data is required for the development of clinical practice recommendations to guide genetic counselling in childhood psychosis.

Conclusion: This case furthers our understanding of presentations of Radio-Tartaglia syndrome. In addition, it highlights the potential utility of rapid genomic sequencing in young children with atypical neuropsychiatric presentations.

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P11.100.C Detection of *AGO2* gene mutation associated with an ultra-rare neurodevelopmental syndrome

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Background: Lessel-Kreienkamp syndrome (LKS) is a rare neurodevelopmental disorder characterized by intellectual disability, global developmental, speech and language delay. Additional features are seizure, hypotonia, gait abnormality, cardiac defects, dysmorphic facial features, behavioural problems. Brain imaging in some patients show white matter abnormalities, thin corpus callosum, enlarged ventricles. LKS is caused by heterozygous mutation in the *AGO2* (Argonaute 2) gene. To date, only 21 patients were reported with LKS worldwide.

Material and Methods: A five-month-old male patient was referred to genetic counseling due to hypotonia, delayed development, feeding difficulties, gastrointestinal problems, failure to thrive, dysmorphic facial features. Later, he exhibited CNS involvement, gait abnormality. Based on MRI findings, the presumed diagnosis was leukodystrophy, therefore a multigene NGS leukodystrophy panel test was carried out, but the result was negative. Since the clinical diagnosis of leukodystrophy was not confirmed by NGS panel, an expanded WES Plus examination was performed.

Results: Exome sequencing analysis identified a c.1092G>A (p.Met364Ile) heterozygous likely pathogenic mutation in *AGO2* gene based on ACMG classification. It has never been reported. Pathogenic mutations in *AGO2* result in associated condition consistent with the phenotype of our patient.

Conclusion: In those cases when the symptoms are well-defined, a multigene panel test may be a useful tool to determine the disease. However, when symptoms are non-specific and multiple organ systems are affected, a more comprehensive genetic examination is more appropriate, especially in neurodevelopmental disorders to identify the genetic abnormality underlying the disease as soon as possible.

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P11.101.D Chronic psychiatric disorder caused by adult-onset metachromatic leukodystrophy

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Background: Neurodegenerative diseases exhibit a broad phenotypic spectrum, often beginning with subtle symptoms that lead to underdiagnoses in early stages. Their heterogeneous etiology represents a diagnostic challenge, especially in light of the therapeutic urgency of early intervention, as seen in rare lysosomal storage disorders such as the adult-onset subtype of metachromatic leukodystrophy (MLD). Most patients are referred

to human genetics at advanced disease stages, when complex motor and cognitive dysfunctions have already developed.

Material and Methods: We established an interdisciplinary framework for the clinical assessment of patients presenting with behavioral and personality abnormalities, integrating early-stage genetic evaluation. Our differential diagnostic approach includes targeted gene or whole-exome sequencing (WES), depending on prior clinical findings. Brain imaging plays a key role in refining the diagnostic focus.

Results: We report on a 42-year-old male patient exhibiting progressive psychiatric symptoms over ten years. His primary manifestations included formal thought disorder, impaired comprehension, mood fluctuations, reduced concentration and mild spastic ataxia. MRI revealed leukoencephalopathy and frontoparietal atrophy. WES identified two heterozygous pathogenic variants c.542T>G; p.Ile181Ser and c.465+1G>A; p.? in the arylsulfatase A (*ARSA*) gene, confirming MLD. We conducted enzymatic analysis and referred the patient to our hematological board to assess eligibility for allogeneic stem cell transplantation.

Conclusion: Although rare, MLD should be considered in young adults with behavioral changes and personality alterations, particularly when specific brain imaging abnormalities are present. An interdisciplinary framework incorporating early genetic testing facilitates timely and accurate diagnosis of heterogeneous underlying causes and may provide access to emerging therapeutic options.

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P11.102.A Transcriptome sequencing reveals aberrant splicing due to novel synonymous *GCDH* variant in Glutaricaciduria Type I

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Background: Synonymous variants can disrupt native splice, introduce de novo splice sites, activate cryptic splice sites, exon skipping, partial exon deletions or intron retention. In some cases, several splicing prediction tools often fail to reliably detect splice alterations due to synonymous variants. Employing transcriptome sequencing is essential to uncover the pathogenicity of such synonymous variants on splicing patterns especially when the clinical phenotype in question is clear.

Material and Methods: A 11-month-old female was suspected with glutaric aciduria type 1 based on clinical manifestations, Tandem Mass Spectroscopy results of elevated blood glutaryl carnitine and MR imaging features of widened sylvian fissures, diffuse cerebral atrophy with bilateral lentiform, caudate, and periventricular T2/FLAIR hyperintensities with diffusion restriction. Exome sequencing identified a homozygous novel synonymous variant NP_000150.1:p.Lys111= in exon 5 of the *GCDH* gene. Transcriptome sequencing was performed to determine the effect of the synonymous variant on splicing.

Results: The NP_000150.1:p.Lys111= synonymous variant [NC_000019.10:g.12892177A>G; NM_000159.4:c.333A>G] is located 2 bp upstream of the consensus donor splice site. The variant was not predicted to disrupt the consensus splice site by any of the splicing algorithms. However, transcriptome sequencing revealed that the synonymous variant disrupted the consensus donor splice site, causing exon skipping, resulting in nonsense mediated decay. The variant was reclassified as pathogenic and submitted to ClinVar (Accession ID: VCV003544377.1).

Conclusion: Our results highlight the need for performing transcriptome sequencing or other approaches for the evaluation of ultra-rare synonymous variants near the splice site, in individuals with a classic phenotype and absence of other significant variants.

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P11.104.C Biallelic *NDUFA9* variants cause a progressive neurodevelopmental disorder with prominent dystonia and mitochondrial complex I deficiency

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Background: Biallelic NDUFA9 variants have been associated with disease in four individuals. Hence, clinicogenetic features of NDUFA9-related disorder remain largely unexplored.

Material and Methods: To delineate the pheno-genotypic spectrum of NDUFA9-related disorder, we screened genetic databases worldwide and collected phenotypic data on individuals with biallelic NDUFA9 variants, which were functionally investigated when possible.

Results: Eight new and four reported cases were identified. Four individuals died by age 13. Neurodevelopmental disorder (NDD) was observed in 90.0% of cases surviving beyond age 4 months. Neurological deterioration always started in the first decade. Among nine affected surviving beyond infancy, major clinical features included dystonia and feeding difficulties/dysphagia/failure to thrive (88.9%), pyramidal involvement (77.8%), moderate-to-severe intellectual disability, seizures and muscle weakness/atrophy (66.7%). All showed basal ganglia MRI signal alterations, with atrophy (50.0%) and swelling (25.0%). We identified five new *NDUFA9* variants and Arg360 as mutational hotspot. Protein modelling suggested that variants cause NDUFA9 misfolding and/or disruption of binding interfaces. Loss of fully assembled complex I with decreased NDUFA9 steady-state levels and/or complex I activity was documented in fibroblasts from three affected.

Conclusion: Our study strengthens the evidence that NDUFA9-related complex I deficiency presents with a broad spectrum of progressive NDD, often accompanied by prominent dystonia, and characteristic Leigh-syndrome MRI pattern.

C.O.I. Disclosure:

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P11.105.D A world-wide study of epilepsy genetic testing in resource rich and resource limited countries - closing the diagnostic and treatment gap

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Background: Advances in precision medicine emphasise the importance of genetic testing in the epilepsies. These developments could increase the gap between resource rich and poor countries. This study aims to determine the access to genetic testing and services and associated challenges worldwide.

Material and Methods: The task force on clinical genetic testing in the epilepsies of the International League Against Epilepsy (ILAE) distributed a global survey between May and Nov 2023 among the ILAE chapters and genetic societies.

Results: 1568 responses were received from 127 countries. Next-Generation sequencing (gene panel/exome sequencing/genome sequencing) was accessible to 90.4% responders in high-income countries as opposed to 16.3% in low-income countries. Personal funding was reported the most common funding source for 37.5% responders in low-income countries as opposed to 8.6% in high-income countries. Consent for genetic testing was obtained by a clinical geneticist for 51% (530/1038) of the responders. It was obtained by a genetic consellor for 26% (270/1038), ranging from 11% in Latin America to 72% in North America. Genetic results were discussed with the family by a clinical geneticist for 63% (652/1041). They were discussed by a genetic counsellor for 37% (385/1041), ranging from 13% in Latin America to 77% in North America.

Conclusion: This global survey show that high cost, funding shortage and poor infrastructure are the source of clear disparity in access to genetic testing across lower income countries. Collaboration in genetic testing initiatives and engagement of local funding bodies in resource-limited countries may be key to addressing these challenges.

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P11.106.A Purinergic receptor gene variants in neurodevelopmental disorders

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Background: Purinergic receptors (P2XRs) are ATP-gated ion channels including seven members (P2X1-7). Their expressions were mainly shown in neurons and immune cells. They have been associated with cancer, mood disorders, neurodegenerative disorders and inflammation-related diseases. P2XRs are important for inflammation processes that are emphasized in neurodevelopmental disorders to uncover etiological factors. Although inflammation processes were emphasized in studies for neurodevelopmental disorders, studies investigating P2XRs are very limited.

Material and Methods: We evaluated variants in P2X receptor genes in patients with neurodevelopmental disorders using clinical and whole exome sequencing data.

Results: In our study, the genetic data of 194 patients diagnosed with neurodevelopmental disorders were determined, and we detected variants of P2X receptor genes in 35 patients diagnosed with intellectual disability, autism spectrum disorder, or epilepsy. We presented detailed clinical findings of cases carrying heterozygous P2RX1 (c.11G>A), P2RX2 (c.943C>T), P2RX3 (c.1165G>A), P2RX4 (c.64C>T, c.427+5G>C, c.1095_1096del, c.964_966del, c.949A>G), P2RX5 (115T>C, c.95T>A, c.274G>A, c.382G>A, c.436+8C>T, c.556G>A c.729C>A, c.928C>T), P2RX6 (c.85A>T) and P2RX7 (c.448G>A, c.462_463del, c.614+1G>A, c.797T>C, c.827G>A, c.886G>A, c.882-60_882-59dup, c.1209T>G, c.1733G>A) gene variants.

Conclusion: COST Action CA21130 "P2X receptors as a therapeutic opportunity (PRESTO)" aims to foster collaboration across European countries for purinergic signaling pathways. Effective pharmacological treatment need is still a big problem for autism spectrum disorders. Suramin is one of the purinergic pathway antagonists and clinical trials are still going on the cases diagnosed with autism spectrum disorder. The available results of these studies are complicated. Testing treatment options in patients carrying specific variants of P2XR genes may be more useful.

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P11.107.B Functional investigation of novel splice-affecting variants in the *SCN9A* gene in patients with Congenital Insensitivity to Pain

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Background: Congenital insensitivity to pain is one of the rarest and most unusual pain disorders, primarily caused by pathogenic variants in the *SCN9A* gene, which encodes the α-subunit of the Nav1.7 voltage-gated sodium channel. To date, most of the described variants in *SCN9A* associated with congenital insensitivity to pain are biallelic frameshifting variants, and the extent to which splice-affecting variants contribute to this rare phenotype remains largely unknown.

Material and Methods: Pathogenic variants in the *SCN9A* gene were identified through WES or WGS. Functional analysis was performed using RT-PCR using patient-derived samples followed by deep next-generation sequencing. Patients were clinically evaluated by expert clinical geneticist to assess genotype-phenotype correlations

Results: WES and WGS identified 4 novel intronic variants in the *SCN9A* gene: c.596+1G>A, c.596+1del, c.2841+5G>A and c.1108-1921G>T located deep in intron 9. Functional analyses demonstrated that all variants affect mRNA splicing, leading to both out-of-frame and in-frame isoforms. The deep intronic variant was shown to lead to inclusion of a pseudo-exon in intron nine leading to a frameshift and formation of a premature stop-codon. Genotype-phenotype analysis did not reveal significant differences in phenotype severity between variants leading to in-frame or out-of-frame transcripts.

Conclusion: Our results suggests that in-frame shortening of the Nav1.7 protein completely disrupts its function in the same manner as frameshifting variants. These findings broaden the understanding of SCN9A-related pain insensitivity and uncover the molecular mechanisms of novel non-coding variants in the SCN9A gene, which is crucial for the development of future tailored therapeutic approaches

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P11.108.C Evaluation of the impact of two homozygous VPS13A mutations in patients with chorea acanthocytosis

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Background: Chorea-acanthocytosis (ChAc) is a severe neurodegenerative disorder with clinical features resembling Huntington's disease, resulting from pathogenic loss-of-function mutations in the VPS13A gene encoding the protein called chorein. In this study, we examined the functional effects of two previously unreported VPS13A homozygous mutations in cases of sporadic neuroacanthocytosis.

Material and Methods: We evaluated the functional impact of two VPS13A mutations identified through exome sequencing: the nucleotide substitution c.9276-2A>T and the 2.8 kb deletion involving exons 69–70 of the gene. In silico tools such as SpliceAI, NNSPLICE, and Netgene2 were employed for the intronic variant to predict the consequences of splicing alterations. The protein expression and in silico characterisation of skin fibroblasts from the ChAc patient with the VPS13A deletion (2.8 Kb), as well as from their relatives and healthy individuals, were assessed.

Results: Our findings revealed that the c.9276-2A>T variant is predicted to modify the highly conserved AG dinucleotide in the intron-69 splice acceptor site, with scores of 0.99 (SpliceAI), 0.97 (NNSPLICE), and 0.38 (Netgene2). Western blot and immunofluorescence analyses on skin fibroblasts demonstrated reduced expression of VPS13A in the affected patient carrying the homozygous deletion of exons 69–70, compared to the mother and control samples.

Conclusion: We described two rare homozygous VPS13A mutations in two cases of suspected ChAc. In silico and functional studies confirmed that these are pathogenic loss-of-function mutations. Our results support the need for regular genetic screening in patients with complex neurological phenotypes, such as neuroacanthocytic syndromes, to allow appropriate genetic counselling and to avoid misdiagnosis.

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P11.109.D A familial case of epilepsy and focal malformation of cortical development suggests a multi-step pathogenic mechanism

Valentina Cetica[1], ***Claudia Mandorlini***[1], *Claudia Bianchini*[1], *Alice Dainelli*[1], *Elena Cellini*[1], *Domenico Rutigliano*[1], *Simona Virdò*[1], *Dalila De Vita*[1], *Daniela Pucatti*[1], *Flavia Fineschi*[1], *Aurelia Iuliana Timis*[1], *Marta Lo Monaco*[1], *Domenica Immacolata Battaglia*[2], *Renzo Guerrini*[1], *Elena Parrini*[1], *Davide Mei*[1]

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Background: Focal malformations of cortical development (MCDs), such as focal cortical dysplasia (FCD) and hemimegalencephaly (HME), are major causes of intractable childhood epilepsy. They are primarily driven by pathogenic variants affecting the mTOR pathway. We describe a family with multiple members affected by focal epilepsy, including two sisters with focal epilepsy and HME.

Material and Methods: We performed next-generation deep sequencing with a UMI-based targeted panel optimized to detect low-level mosaic variants, in brain-derived DNA from a 5-year-old girl with focal epilepsy and HME. We then analyzed all affected family members.

Results: In the proband's brain-derived DNA, we identified a low-level mosaic MTOR variant (p.Cys1483Tyr, 1% VAF), absent in her blood and a heterozygous NPRL3 deletion which segregated in all family members affected by focal epilepsy, including her older sister with HME. The DNA analysis of paraffin-embedded brain tissue of the older sister excluded the MTOR variant but did not allow a complete analysis of MTOR pathway genes, leaving the presence of a second-hit unresolved.

Conclusion: This family, presenting with familial focal epilepsy and HME in two sisters, suggests a novel pathogenic mechanism underlying the development of focal MCDs. Specifically, the heterozygous deletion of NPRL3 may prompt a second somatic event, which is essential for the structural malformation. We hypothesize that a second somatic variant was also present in the older sister but remained undetected due to poor DNA quality. Future studies on patients with HME could help to validate this hypothesis.

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P11.110.A The clinical and molecular spectrum of Neurodegeneration with Brain Iron Accumulation in the largest national cohort

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Background: Neurodegeneration with brain iron accumulation (NBIA) is an ultra-rare group of neurological disorders characterized by progressive dystonia, dysarthria, spasticity, neuropsychiatric abnormalities, and iron deposition in the basal ganglia. Due to its rarity, only a limited number of single-gene cohorts have been reported. The most prevalent clinical form of NBIA remains unclear, with some sources suggesting PKAN and others BPAN. In Russia, the NBIA spectrum within a large national registry remains undetermined.

Material and Methods: This study utilized retrospective analysis of DNA tests from the two largest Russian medical genetics centers (2017–2024). Over 7,000 whole-genome sequencing (WGS) samples were analyzed for variants in nine NBIA-related genes (*WDR45, C19orf12, PANK2, PLA2G6, FA2H, COASY, CP, FTL, ATP13A2*). Variants were annotated using wANNOVAR, and potential splice-affecting variants were predicted with SpliceAI. Clinical significance was assessed following ACMG/AMP guidelines.

Results: The study identified 114 patients with 74 unique variants across nine NBIA-related genes. SpliceAI predicted 20 variants (27%) as splice-disrupting. BPAN was the most common

subtype (34%), followed by MPAN (32%) and PKAN (16%). Pathogenic variants were found in 50.4% of cases, likely pathogenic in 27%, and variants of uncertain significance (VOUS) in 22.7%. Milder clinical forms associated with *WDR45* and *C19orf12* genes were identified for the first time.

Conclusion: This research describes the largest national NBIA cohort to date, revealing a distinct NBIA spectrum in Russia, with BPAN and MPAN comprising two-thirds of cases, differing significantly from global patterns.

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P11.111.B *A homozygous NAV3 variant further strengthens links to neurodevelopmental disorder*

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Background: Neurodevelopmental disorders (NDDs) represent a diverse and heterogeneous group of conditions, including neurodevelopmental delay, autism spectrum disorder (ASD), and neurodevelopmental encephalopathy with epilepsy (NDEE). While these disorders often share phenotypic similarities, their underlying genetic causes can vary widely, complicating clinical diagnosis.

Methods: In this study, we performed whole-genome sequencing (WGS) on a family with autosomal recessive inheritance of indeterminate neurological symptoms. The proband (II-2) underwent WGS, followed by variant filtering using an in-house bioinformatics pipeline. Sanger sequencing was employed to confirm the identified variants. Furthermore, 3D protein modeling was performed for structural predictions, and RT-qPCR analysis was conducted to assess gene expression.

Results: A novel biallelic missense variant in NAV3 (c.3430T>C; p.Ser1144Pro) was detected, segregating with the phenotype in the family. Subsequent 3D protein modeling revealed significant alterations in the structure of the NAV3 protein, and RT-qPCR analysis showed a decrease in the expression of the NAV3 gene compared to the control and other healthy family members, suggesting a potential pathogenic effect.

Conclusion: This finding further implicates NAV3 in neurodevelopmental processes, confirming its role in autosomal recessive neurodevelopmental disorders. Notably, this is the third report linking NAV3 mutations to NDDs. Further research, including functional studies and the identification of additional cases, is needed to fully understand the role of NAV3 in human neurodevelopmental disorders.

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M. Umair: None. **M. Alfadhel:** None.

P11.112.C Clinical and Genetic Spectrum of Syndromic Epilepsies: Insights from a Cohort in a Highly Consanguineous Population

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Background: Syndromic epilepsies exhibit significant clinical and genetic heterogeneity, requiring high-throughput sequencing for accurate diagnosis. In populations where sequencing is not routinely available, the genetic landscape remains insufficiently characterized. This is particularly relevant in highly consanguineous populations, where the incidence of autosomal recessive disorders is higher.

Material and Methods: We conducted a clinical and genetic study on patients with syndromic epilepsy followed at the Genetics Department of Mongi Slim Hospital, Tunis, with at least one index case per family undergoing exome sequencing (ES).

Results: We analyzed 37 patients from 34 families with a consanguinity rate of 35%. Developmental delay and/or intellectual disability were present in 94%, and facial dysmorphisms and congenital anomalies in 68% and 86%, respectively. Seizures manifested early, with a mean onset at just 10 months, and a concerning 22% of cases presenting in the neonatal period. Neurological comorbidities were found in 73% of cases, and nearly half had drug-resistant epilepsy. ES identified 35 variants, including 32 SNVs and 3 CNVs, with a definitive genetic diagnosis in 81% of cases. Notably, 30% of pathogenic variants were in actionable genes.

Conclusion: This study provides the most comprehensive genetic landscape of syndromic epilepsy in a Tunisian cohort. Understanding the genetic makeup of North African populations is crucial, as they are an integral part of European society. Expanding genomic studies to underrepresented populations will not only enhance local diagnostic capabilities but also contribute to global scientific advancements by refining gene-disease correlations and broadening the mutational spectrum of epilepsy-related genes.

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P11.113.D Unraveling the genetic landscape of corpus callosum malformations (CCM) and autism spectrum disorders: Toward precision diagnosis

Bochra Khadija[1,2,3], *Wafa Dahleb[1], Hamza Hadj abdallah[1], Wafa Slimani[1], Ayda Bennour[1,2], Adnen Mlika[4], Najla Soyah[5], Khouloud Rjiba[1], Dr Attia[6], Ali Saad[1,2], Christel Depienne[7], Soumaya Mougou-Zerelli[1,2]*

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Background: Corpus callosum malformations (CCM) are common congenital brain anomalies, ranging from complete or partial agenesis to hypoplasia or dysplasia. These malformations may occur isolated or alongside other conditions and are frequently associated with neurodevelopmental disorders, particularly autism spectrum disorders (ASD). The clinical and genetic heterogeneity of CCM complicates genotype-phenotype correlations, especially in prenatal diagnosis.

Material and Methods: This study, conducted on a cohort of patients from Farhat Hached university hospital in Sousse (2010–2024), employing cutting-edge techniques like array CGH and exome sequencing.

Results: Our research focused on six patients with CCM and ASD, uncovering significant genetic abnormalities in three cases. Array CGH revealed two critical copy number variations (CNVs) in two patients: a 3.69 Mb deletion in 1p32 involving the *NFIA*, a key regulator of gliogenesis and callosal development and a 1,8 Mb duplication in 1q31.1 affecting *BRINP3* implicated in neurodevelopmental pathways. Exome sequencing further identified a double variant in *WLS* and *ERCC8*, linked to Zaki and Cockayne syndromes, respectively. Additionally, a variant in *MAPK8IP3*, a gene crucial for axonal transport and neuronal connectivity, was discovered, reinforcing its role in CCM and ASD pathogenesis. These findings highlight the involvement of these genes in key neurodevelopmental processes, providing novel insights into CCM and ASD pathogenesis.

Conclusion: The integration of array CGH, exome sequencing, psychiatric assessments, brain imaging and functional studies provides a complete understanding of the genetic bases of CCM and ASD. This multidisciplinary approach promises improved diagnosis, clinical management, and future developments in prenatal care and genetic counseling.

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P11.114.A Autism spectrum disorder in a child carrying a novel truncating variant in *DIP2B*, a gene previously associated with repeat expansion disorder

Gioia Mastromoro[1], *Martina Rinelli*[2], *Nader Khaleghi Hashemian*[3], *Daniele Guadagnolo*[3], *Daniela Miraglia*[3], *Antonio Pizzuti*[3], *Mario Mastrangelo*[3], *Angelo Minucci*[2], *Mauro Ciro Antonio Rongioletti*[1]

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Background: Autism spectrum disorder includes a group of conditions that may vary based on their association with additional clinical features. Although it is a multifactorial condition, in some cases causative variants of neurodevelopmental alterations and autism have been found.

Material and Methods: A 8-year-old male child was referred for motor stereotypies and mannerisms of the distal extremities of the upper limbs. Cognitive and behaviour reassessment showed an autism spectrum disorder without intellectual disabilities and without language disorders. Standard karyotype, chromosomal microarray analysis and exome sequencing were requested.

Results: Cytogenetic assays did not detect imbalances. Exome sequencing revealed the de novo nonsense c.265C>T; p.(Arg89-Ter) variant in the *DIP2B* (MIM *611379) brain-expressed gene. The loss of a functional *DIP2B* allele, caused by promoter methylation in presence of a CGG expansion in the 5-prime untranslated region, has been associated with a form of neurodevelopmental disorder with autosomal dominant inheritance, related to the FRA12A fragile site (MIM #136630). The DIP2B protein contains a DMAP1-binding domain, involved in DNA-methylation processes. The affected cases reported in literature show the methylated repeat expansion, exhibiting low mRNA levels, while unaffected carriers with unmethylated less elongated expansion presented increased gene expression. We propose that rare truncating variants in *DIP2B* might exert a loss-of-function effect comparable to the large methylated expansions.

Conclusion: This is the first case of a child with autism spectrum disorder detected with a novel truncating variant in *DIP2B*, showing a different molecular basis in a gene previously considered to cause a repeat expansion disorder.

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P11.115.B Patients with complex and very-early-onset *ATL1*-related spastic paraplegia offer insights on genotype/phenotype correlations and support for autosomal recessive forms of SPG3A

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Background: Spastic paraplegia type 3A (SPG3A) is the second most common form of hereditary spastic paraplegia (HSP). This autosomal-dominant-inherited motor disorder is caused by heterozygous variants in the *ATL1* gene which usually presents as a pure childhood-onset spastic paraplegia. Affected individuals present muscle weakness and spasticity in the lower limbs, with symptom onset in the first decade of life. Individuals with SPG3A typically present a slow progression and remain ambulatory throughout their life.

Material and Methods: Here we report three unrelated individuals presenting with very-early-onset (before 7 months) complex, and severe HSP phenotypes (axial hypotonia, spastic quadriplegia, dystonia, seizures and intellectual disability).

Results & Conclusion: For 2 of the 3 patients, these phenotypes led to the initial diagnosis of cerebral palsy (CP). These individuals carried novel *ATL1* pathogenic variants (a de novo *ATL1* missense p.(Lys406Glu), a homozygous frameshift p.(Arg403Glufs*3) and a homozygous missense variant (p.Tyr367-His)). The parents carrying the heterozygous frameshift and missense variants were asymptomatic. Through these observations, we increase the knowledge on genotype-phenotype correlations in SPG3A and offer additional proof for possible autosomal recessive forms of SPG3A, while raising awareness on these exceptional phenotypes. Their ability to mimic CP also implies that genetic testing should be considered for patients with atypical forms of CP, given the implications for genetic counselling.

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P11.116.C Clinical characteristics and mutation spectrum of Neurofibromatosis Type 1 in a North Indian cohort

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Background: Neurofibromatosis type 1 (NF1) is a unique, highly penetrant neuro-cutaneous disorder with a wide range of manifestations. Though the clinical diagnosis of NF1 is straight forward, there can be other disorders which mimic NF1, especially its cutaneous features which can be a diagnostic dilemma in certain conditions. Here we describe the clinical and mutation spectrum of a series of individuals whose primary diagnosis was NF1 or NF1 related disorders.

Material and Methods: We have screened 31 consecutive unrelated individuals who fulfilled the clinical criteria of NF1. Whole exome sequencing (WES) was done in all individuals except one with suspected microdeletion syndrome with NF1 in whom Cytogenetic microarray (CMA) was done. All the variants were classified as per ACMG guidelines.

Results: Out of 31 suspected patients, 27 had germline pathogenic/likely pathogenic variants involving NF1 gene. Five novel and 22 known variants in coding and non-coding regions were identified, among them 7 variants were deletions (22.6%), 7 nonsense (22.6%), 4 splice-site (12.9%), 4 missense (12.9%), 3 duplications (9.6%) and 2 (6.5%) were contiguous deletions. In those where NF1 variants were not detected, 3 had neurofibromatosis type 2 (NF2) and 1 rare autosomal recessive form of Elher Danlos syndrome.

Conclusion: We hereby present the wide range of manifestations in different age groups and the mutation spectrum ranging from small scale variants to contiguous gene deletion syndromes involving NF1 gene. We highlight the usefulness of molecular testing and its importance in tumor surveillance and genetic counseling in this disorder.

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P11.117.D Chromosomal microarray analysis in patients with developmental delay/intellectual disability and autism spectrum disorder: a retrospective study

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Izmir City Hospital, Izmir, Turkey.

Background: Developmental delay (DD), intellectual disability (ID), and autism spectrum disorder (ASD) are among the most prevalent conditions within the spectrum of rare diseases. Chromosomal microarray (CMA) technology has been widely utilized as a diagnostic tool in patients with DD/ID and ASD. For many years, CMA has been employed as a first-tier genetic test to elucidate the etiology of these disorders and continues to maintain its diagnostic utility. In this study, we aimed to retrospectively evaluate the chromosomal microarray results of 319 patients (age range:6 months to 28 years) with DD/ID and ASD to understand the effectiveness of the CMA testing in clarifying the etiology.

Material and Methods: Chromosomal microarray analysis was performed on 319 patients (216 males, 103 females) using Applied Biosystems CytoScan™ HT-CMA® Assay kit. Identified copy number variations (CNVs) were analyzed using Chromosome Analysis Suite (CHAS 4.5-NetAffx 2024–0101 version) based on the GRCh38 (hg38) reference genome.

Results: 132 CNVs were reported in 32.9% of patients (n = 105). Among the reported CNVs (n = 132), 29.5% (n = 39) were classified as pathogenic or likely pathogenic while 70.5% (n = 93) were considered variant of uncertain significance (VOUS). While 74.3% (n = 29) of the pathogenic/likely pathogenic CNVs were heterozygous deletions, 25.7% (n = 10) were heterozygous duplications.

Conclusion: Our study indicates the ongoing diagnostic value of chromosomal microarray analysis in elucidating the genetic etiology of DD/ID and ASD, demonstrating its continued relevance in medical genetics practice.

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P11.118.B CARE4ARID1B: Developmental Trajectories in ARID1B-Related Disorder - a Multi-Method Multi-Site Prospective Natural History Study

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Background: ARID1B is one of the most frequently mutated genes in monogenic neurodevelopmental disorders (NDDs), with an approximate prevalence of one in 9,500 individuals. ARID1B-related disorder (ARID1B-RD) manifests in early childhood as moderate to severe developmental delay while older individuals have intellectual disability (ID) and often autism spectrum disorder alongside other clinical signs. The current understanding of the disorder's developmental trajectories is strikingly limited.

Material and Methods: The project will address this gap by employing a prospective longitudinal multi-method design. Its primary objectives are twofold: to establish a comprehensive, integrative understanding of the development of ARID1B-RD encompassing all relevant functional domains and physiological systems and to characterize traditional and novel clinical endpoints for upcoming targeted clinical trials. To attain these objectives, we will monitor the development of 135 children and adolescents with ARID1B-RD in seven sites, for 30 months. Monitoring will involve bi-annual standardized NDD assessments, caregivers' reports, ARID1B-RD-specific biomarkers (EEG, episignatures, proteomics and facial phenotype), and collection of ecologically valid data based on caregivers' reports and wearables tapping into motor and language development. The project team brings a diverse set of complementary expertise and collaborates with patient advocacy organizations.

Results: We will present the study design, a call for patients, and preliminary data collected from the first participants.

Conclusion: The data will allow identifying and cross-validating clinical endpoints, and serve as an external historical control, thus facilitating the development of effective therapeutics. Moreover,

the methods implemented will serve as an example for future multinational/lingual natural history studies for NDDs.

C.O.I. Disclosure:

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P11.119.A Identifying clinically meaningful subgroups in Autism Spectrum Disorders: linking phenotypic profiles to genetic insights

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Background: Autism Spectrum Disorders (ASDs) are highly heterogeneous and complex neurodevelopmental conditions that are challenging to characterize and accurately diagnose.

Identifying clinically meaningful subgroups of ASDs could improve our understanding of the conditions and lead to more personalised approaches in treatment and care. In this study, we explore the existence of distinct ASDs subgroups using phenotypic data and investigate the association between these subgroups and distinct genetic profiles.

Material and Methods: To achieve this, we build a patient similarity network and apply Louvain community detection to stratify participants from the Simons Foundation Powering Autism Research for Knowledge study. We focus on diagnostic questionnaire data from the basic medical screening, and summary scores from the Developmental Coordination Disorder Questionnaire, Social Communication Questionnaire, and the Repetitive Behaviour Scale-Revised. We apply the same methods to the Simons Simplex Collection cohort and compare our stratification results across datasets. We explore the correlation between genetic markers and identified phenotypic profiles.

Results: Our analyses reveal multiple ASDs subgroups with distinct phenotypic and genotypic profiles. Traditional questionnaire summary scores fail to capture the necessary granularity for effective ASDs stratification, while specific questionnaire data and detailed clinical variables offer a more nuanced understanding of ASDs heterogeneity.

Conclusion: The research findings have the potential to refine the characterisation of ASDs, aiding improved diagnosis and supporting the development of targeted interventions. Understanding the phenotypic and genetic underpinnings of these subgroups could ultimately lead to improved outcomes for individuals with ASDs, advancing the shift towards more personalised clinical support for individuals.

C.O.I. Disclosure:

H. Issa: None.

P11.120.C Microcephaly and simplified gyral pattern due to compound heterozygous variants in *TUBGCP6*: Prenatal and postnatal findings in a multiplex family

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Background: Microcephaly and chorioretinopathy have been associated with bi-allelic variants in *TUBGCP4, TUBGCP6* and *PLK4*, as well as monoallelic variants in *KIF11*. Since the implication of biallelic variants in *TUBGCP6*, in 2012, ~15 affected individuals have been reported, including two unborn fetuses.

Methods: We sought to establish the molecular diagnosis in a family with two pregnancies affected by microcephaly, delayed sulcation and additional findings on brain imaging. Prenatal quatro exome sequencing (ES), and subsequently single genome sequencing of an affected female child, were pursued.

Results: A non-consanguineous couple presented during their first pregnancy due to fetal microcephaly, delayed sulcation, short and thick corpus callosum and small cerebellum. The pregnancy was terminated, and genetic evaluation- including Chromosomal Microarray Analysis (CMA) and fetal trio ES- were nondiagnostic. Upon a subsequent pregnancy, similar findings in the female fetus were noted, and following close and extensive prenatal follow up, including three fetal brain MRIs, the parents decided to complete the pregnancy. Prenatal ES demonstrated a maternally-inherited heterozygous c.1630del, p.Val544CysfsTer10 variant in *TUBGCP6* shared by the two affected fetuses, however a second variant was not detected. At 2 years of age, the female child shows microcephaly (−3.5 SD), however continues to achieve developmental milestones within the normal range. Ophthalmologic exam shows astigmatism and hyperopia, but no chorioretinopathy. Single genome sequencing revealed a second intronic c.2271-125G>A variant in *TUBGCP6*, confirming the diagnosis in the family.

Conclusions: Our experience with this multiplex family expands the current knowledge regarding the mutational spectrum and neurodevelopmental phenotype of *TUBGCP6*-associated tubulinopathy.

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H. Shani: None. **E. Kassif:** None. **M. Barkenstadt:** None. **E. Hadi:** None. **O. Bar Yosef:** None. **B. Pode Shakhed:** None.

P11.122.A In-depth behavioral characterization of a rat model of Schaaf-Yang syndrome

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Background: Schaaf-Yang syndrome (SYS) is a rare neurodevelopmental disorder caused by truncating variants in the maternally imprinted *MAGEL2* gene. It is characterized by autism spectrum disorder, joint contractures, hypotonia and feeding difficulties. This study provides a behavioral characterization of a novel rat model ("*Magel2 P mut* rats") carrying a truncating mutation on the paternal allele of *Magel2*, which offers greater construct validity for SYS compared to previous animal models that focused on complete *Magel2* deletion.

Material and Methods: We conducted a comprehensive battery of eight different behavioral tests including advanced behavioral assessment systems, such as LABORAS, a non-invasive system for monitoring rodent behavior in the home cage, ultrasonic vocalization (USV) measurements, and CatWalk XT, a precise tool for locomotion analysis.

Results: *Magel2 P mut* rats showed several significant alterations in isolation-induced USVs compared to wild-type littermates. Despite lower body weight in early life, *Magel2 P mut* rats spent significantly more time eating and drinking, as revealed by home-cage monitoring. Additionally, *Magel2 P mut* rats displayed decreased anxiety-like behavior in the elevated plus maze test, delayed decision-making in the T-maze spontaneous alternation

task, several phenotypes of abnormal social interaction and irregular gait patterns.

Conclusion: This study provides new insights into the phenotype of a novel rat model for SYS and provides behavioral outcomes relevant for intervention. The observed behavioral alterations, particularly in social interaction, communication, feeding behavior, and locomotion, may reflect symptoms seen in SYS, underscoring the value of this model for preclinical research on SYS pathophysiology and potential treatments.

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P11.123.B *KIF11*-related microcephaly masquerading as congenital CMV: a diagnostic journey

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Background: Microcephaly with or without chorioretinopathy, lymphedema, or impaired intellectual development (MCLMR, OMIM #152950) is a microcephaly syndrome presenting with variable phenotypic features. The most common clinical findings are mild to severe microcephaly and chorioretinal anomalies. This disorder is caused by variants in *KIF11* (Kinesin Family Member 11, OMIM *148760), located on chromosome 10q23. In this study, we present a patient who was born with microcephaly and received valganciclovir at 2 months of age due to positive CMV serology, but was later found to have a pathogenic *KIF11* variant through molecular analysis at 15 months of age.

Material and Methods: Whole exome sequencing (WES) was performed, as it was not possible to definitively distinguish between congenital and acquired CMV infection.

Results: WES analysis identified a heterozygous c.2548-1G>A splice-affecting variant in the KIF11 gene. The detected variant was classified as "pathogenic" according to the ACMG guidelines and was not reported in healthy population databases.

Conclusion: This case highlights the importance of considering genetic etiologies in patients with congenital microcephaly, even when an initial infectious cause, such as congenital CMV, has been identified.

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P11.124.C A novel de novo *KCND2* variant emphasizes the pathogenic relevance of a three aminoacid mutation hotspot

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Background: The *KCND2* gene (MIM*605410) encodes the voltage-gated potassium channel Kv4.2, which facilitates transmembrane potassium transport in excitable membranes, primarily within the brain. To date, only 6 pathogenic *KCND2* variants have been reported, all associated with global developmental delay and variants in specific residues in transmembrane segment S6. Symptoms typically manifest within the first six months of life and include hypotonia, seizures/epilepsy, visual impairments, delayed motor development, speech and cognitive deficits, and autism spectrum disorder.

Material and Methods: We performed whole-exome sequencing trio analysis on a 10-month-old male patient. He was delivered via cesarean section with an Apgar score of 9. His early development was unremarkable; however, by three months of age, his parents reported stagnation in developmental progress, loss of previously acquired skills, absence of object grasping, lack of eye contact and visual fixation, and no response to auditory stimuli despite normal hearing test results. A medical evaluation revealed significant axial hypotonia, suspected decreased visual acuity, nonspecific delayed cerebral myelination on MRI, and abnormal sleep architecture with generalized changes on EEG. No epileptic seizures were observed.

Results: WES trio analysis identified a de novo *KCND2* variant, p.(Pro403Thr) (NM_012281.3:c.1207C>A).

Conclusion: All six previously reported pathogenic *KCND2* variants, along with our identified variant, are missense variants affecting amino acids V402, P403, and V404 - a region critical for channel gating. The findings in our patient further support the association between *KCND2* missense variants within this critical region (specifically at amino acids 402–404) and global developmental delay.

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P11.125.D MELAS syndrome in Hungary: insights from clinical characteristics and longitudinal follow-up

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Background: MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Episodes) syndrome is a mitochondrial DNA disease characterized by a systemic disturbance in oxidative phosphorylation. The disease exhibits a wide clinical spectrum and significant phenotypic variability, even within families. The estimated global prevalence of m.3243A>G-associated MELAS varies widely (0.2–236/100,000). Diagnostic and therapeutic challenges are compounded by the broad symptom spectrum and the fluctuating nature of the disease.

Material and Methods: We aimed to describe the clinical characteristics of MELAS patients diagnosed with the rare m.3243A>G variant in Hungary. We investigated intrafamilial phenotypic variability, the correlation between heteroplasmy levels and disease onset, and the relationship between symptom progression and patient outcomes. Data were collected from 27 patients (9 males, 18 females) across 17 families. In addition to deep phenotyping and clinical assessment, longitudinal follow-up data from 18 patients were analyzed over a mean period of 5.99 (±4.41) years.

Results: The mean age of symptom onset was 33 (±13.21) years, with five childhood cases. Genetic diagnosis was confirmed in 24 cases using blood-derived DNA and in three using muscle-derived DNA. The mean heteroplasmy rate from blood-derived DNA was 32.46% (±14.69%). The average diagnostic time was 7.15 (±6.61) years. Apart from primary myopathic symptoms, initial manifestations primarily included well-established comorbidities

such as diabetes and hearing impairment, even in patients with heteroplasmy as low as 15%.

Conclusion: Our cohort's phenotypic spectra, combined with clinical data offer valuable insights to refine diagnostic markers, optimize patient pathways, and long-term prognosis.

C.O.I. Disclosure:

I.J. Jimoh: None. **P. Balicza:** None. **A. Gal:** None. **M.J. Molnar:** None.

P12 Neuromuscular Disorders

P12.001.A From Newborn Screening to Functional Insight: *SMN1* VUS studies in zebrafish supported therapeutic decisions

Brunhilde Wirth[1], *Brett W. Stringer*[2], *Yougang Zhang*[2], *Afsaneh Taghipour-Sheshdeh*[2], *Sebastian Zetzsche*[1], *Valentina Piano*[1], *Nico Fuhrmann*[1], *Jutta Becker*[1], *Mert Karakaya*[1], *Shuxiang Goh*[3], *Heike Kölbel*[4], *Michelle Farrar*[5], *Jean Giacomotto*[2]

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Background: Newborn screening (NBS) in spinal muscular atrophy is widely implemented, enabling early detection and immediate treatment for newborns with homozygous *SMN1* deletions, providing significant neurological benefits. A PCR-based NBS assay with dried blood spots, identify ~95% of cases by absence of exon 7 *SMN1* signals, confirmed via MLPA testing, which also determines *SMN2* copy numbers critical for therapy. Remaining cases involve subtle *SMN1* mutations undetected by screening, often diagnosed too late for prevention.

Results: This study highlights two newborns from Germany (P1) and Australia (P2), identified as lacking *SMN1* signals via NBS. However, MLPA revealed each had one *SMN1* copy; moreover, P1 had no *SMN2* copies, and P2 had one *SMN2* copy. Sanger sequencing uncovered two novel *SMN1* variants of unknown significance (VUS) in exon 7, resulting in the same frameshift mutation, p.(Arg288Alafs*5). These novel variants caused false-negative PCR results due to primer annealing issues. The Australian Functional Genomics Network supported us in developing a zebrafish assay to functionally analyze these VUS. In vitro-transcribed mRNAs from 861VUS and 855VUS were tested alongside positive and negative controls. Animals lacking *Smn* or expressing a pathogenic variant developed severe malformations and died by 6 day-post-fertilization. Contrary, both VUS mRNAs, like wild-type and a non-pathogenic variant, rescued zebrafish SMA hallmarks, including morphology, swimming ability, and survival. Both children, now one-year-old, remain asymptomatic.

Conclusion: This study validates zebrafish as a tool for VUS analysis, supporting diagnostic and therapeutic decisions in clinical practice. Most importantly, therapeutic costs exceeding US$2 million per child have been avoided.

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P12.002.B In vivo modeling and characterization of a novel *SNUPN*-deficiency-associated muscular dystrophy (Award Candidate)

Hilal Pırıl Saraçoğlu[1,2], *Marwan Nashabat*[1,2], *Burak Sarıbaş*[1,2], *Elanur Yılmaz*[2], *Şeymanur Tur*[1,2], *Duygu Naz Kutlu*[2], *Emre Yakşi*[2,3], *Hülya Kayserili*[4,5], *Nasrinsadat Nabavizadeh*[1,2], *Nathalie Escande-Beillard*[1,2]

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Background: We recently identified *SNUPN* loss-of-function mutations as the genetic cause of a novel form of muscular dystrophy (MD), expanding the spectrum of limb-girdle MDs (MIM#620793). Snurportin-1 (SPN1), the protein encoded by *SNUPN*, is crucial for the nuclear import of spliceosomal small nuclear ribonucleoproteins (snRNPs), yet its physiological role in muscle homeostasis remains poorly understood. To address this, we developed a *snupn* zebrafish mutant model, leveraging the high evolutionary conservation between humans and zebrafish, the absence of *snupn* duplication in zebrafish and more importantly their similar neuromuscular systems.

Material and Methods: *snupn* mutant zebrafish line was generated using CRISPR/Cas9. Validation and characterization were performed via Sanger sequencing, qPCR, immunofluorescence, histology, electron microscopy, confocal imaging, birefringence, and behavioral analyses.

Results: The *snupn* mutant zebrafish line harbours an 8-bp insertion, resulting in a truncated Spn1 protein (p.Ser116Ter) and reduced *snupn* mRNA levels in larvae and muscle tissue. Histological and ultrastructural analyses revealed muscle fibre abnormalities and subcellular organelle alterations. Reduced birefringence and phalloidin staining indicated impaired muscle fiber integrity, correlating with significantly reduced locomotor activity. Additionally, Spn1 mutants showed marked reduction in laminin and β-DAG, consistent with extracellular matrix disorganization observed in patient muscle samples. Further analyses revealed Spn1 is essential for proper muscle fiber differentiation and specification.

Conclusion: The Spn1-deficient zebrafish model faithfully recapitulates the human disorder, providing a valuable tool for investigating disease pathogenesis and developing therapeutic strategies to mitigate muscle defects in patients.

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P12.003.C Mutational screening of myopathy gene ACTN2 reveals an aggregation hot-spot in the actin-binding domain (Award Candidate)

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Background: *ACTN2* encodes alpha-actinin-2, a structural protein expressed in both cardiac and skeletal muscle sarcomeres. The primary function of the protein is to cross-link actin in the sarcomere Z-disk. The gene has a long-standing association with cardiomyopathy, but recently, it has been shown to cause skeletal muscle disease as well. However, with small number of patients and limited understanding of the underlying molecular mechanisms, variant interpretation in *ACTN2*-related diseases is currently challenging.

Material and Methods: One of the few clearly demonstrated disease mechanisms of *ACTN2* is aggregation of mutated protein. Using a cellular model and over 200 GFP-tagged *ACTN2* constructs, we screened for aggregating variants in various domains of the gene.

Results: We found nearly 20 positions with aggregating variants in the actin binding region, more specifically in the interface between its two calponin homology domains. Additionally, a few positions with aggregating variants were identified in the neck region of the protein. In contrast, no aggregating variants were found in the EF34-domain. These results suggest that the interface between the two calponin homology domains of the actin binding region is susceptible to aggregation causing variants.

Conclusion: The results indicate that aggregation screening may be a useful initial test to assess the pathogenicity of variants of uncertain significance in the actin binding region of alpha-actinin-2. This mutational screening study will, together with further functional work characterizing other possible molecular disease mechanisms, ease *ACTN2* variant interpretation.

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J. Ranta-aho: None. **P. Jonson:** None. **J. Sarparanta:** None. **B. Udd:** None. **M. Savarese:** None.

P12.004.D Exploring morphological and functional biomarkers in iPSC-derived corticospinal motor neurons of infantile-onset ascending hereditary spastic paralysis

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Background: Infantile-onset Ascending Hereditary Spastic Paralysis (IAHSP, #607225) is a rare (<1:100.000) autosomal recessive Motor Neuron Disease (MND) diagnosed in just over 50 children, characterized by severe spasticity of the lower limbs in early childhood, which worsen to tetraplegia, anarthria, dysphagia, and slow eye movements. IAHSP is due to mutations in the ALS2 gene (MIM#606352, 2q33.1) encoding Alsin, a protein mainly expressed in the central nervous system with a key role in cytoskeleton integrity and neurite outgrowth. ALS2 mutations lead to a loss of function of Alsin, resulting in progressive degeneration of upper motor neurons (UMNs). A human in vitro model from ALS2 patients has never been developed.

Material and Methods: Using induced pluripotent stem cell (iPSC) technology, patient-specific neuronal models were created from four IAHSP cases: two unrelated girls with compound heterozygous mutations (NM_020919.3:[c.4368delG];[c.4831C>T] and NM_020919.3:[c.529G>C];[c.3304G>A]), and a pair of siblings with homozygous mutations NM_020919.3:[c.4368delG];[c.4368delG].

Results: IPSC were differentiated into corticospinal motor neurons (CSMN) expressing Ctip2 and Znf312 (V layer markers). IF and Western blot on IAHSP mature neurons revealed a decrease in Alsin expression. Morphological examination of CSMNs showed a reduced average lengths in patient-derived models compared to controls. Electrophysiological analysis of neurons from one IAHSP patient revealed altered excitability. Specifically, current clamp recordings showed reduced input resistance, increased rheobase, and lower firing frequency. Patch-clamp studies are ongoing for the other IAHSP cases.

Conclusion: These findings identify potential biomarkers and therapeutic targets, offering insight into disease mechanisms and paving the way for personalized treatments.

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P12.005.A Comprehensive Genotypic, Phenotypic, and Biochemical Characterization of GOT2 Deficiency: A Progressive Neurodevelopmental Disorder with Epilepsy and Abnormal Movements (Award Candidate)

H. M. German[1], *M. Zaki*[2], *M. Usmani*[3,4,5], ***N. Chamanrou***[6,7,8], *I. Karagoz*[9], *S. Efthymiou*[9], *M. Abdel-Hamid*[2], *H. Abdelhafez Arabiyat*[10], *A. Ghaffar*[3,11], *M. Shahzad*[5], *H. van Bokhoven*[12], *Z. Ahmed*[3,13], *O. Yaghini*[14], *N. Hosseini*[15], *M. Majidinezhad*[15], *S. Alavi*[16], *M. Bosma*[1], *M. Broeks*[1], *D. Türdoğan*[17], *M. Suri*[18], *L. Laura de Godoy*[19], *N. Verhoeven-Duif*[20], *S. Riazuddin*[5,21], *J. Gleeson*[22,23], *C. Alves*[24], *J. Jans*[1], *S. Riazuddin*[3], *H. Houlden*[9], *R. Maroofian*[9];

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Background: Glutamic-oxaloacetic transaminase (GOT), also known as aspartate aminotransferase, catalyzes the reversible transamination of oxaloacetate and glutamate to aspartate and α-ketoglutarate. Two isoforms, cytosolic (GOT1) and mitochondrial (GOT2), are integral to the malate-aspartate shuttle (MAS), a key regulator of intracellular redox homeostasis. Recently, five patients with bi-allelic variants in GOT2 were described, presenting with developmental and epileptic encephalopathy.

Material and Methods: We report 11 additional patients with homozygous *GOT2* variants, along with additional data from 4 previously reported patients. Through genetic, clinical and biochemical analyses, we further characterize the phenotypic spectrum of GOT2 deficiency.

Results: Most patients exhibited progressive neurodevelopmental delay, severe to profound intellectual disability, infantile epilepsy, progressive microcephaly, and hypotonia evolving into spasticity with axial hypotonia. Dysmorphic features included narrow foreheads, broad nasal tips, and tall or pointed chins. Neuroimaging revealed two severity groups based on cerebral volume loss and myelination defects. Thinning of the corpus callosum and white matter abnormalities were common. Biochemical profiling identified low aspartate and high glycerol-3-phosphate in dried blood spots as potential biomarkers. Patient fibroblast cells showed reduced serine and glycine biosynthesis, rescuable by pyruvate supplementation.

Conclusion: These findings expand the phenotypic spectrum of GOT2 deficiency, establish it as a cause of DEE, and propose novel biomarkers for diagnosis and treatment.

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P12.006.B Long-read sequencing reveals the genetic origins of SMA alleles and excludes whole-blood DNA methylation as an epigenetic biomarker

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Background: The complex 2 Mb SMN locus on chromosome 5q13, containing the SMA-causing SMN1 gene and its modifier SMN2, remains unresolved due to segmental duplications. SMN2 copy number, influenced by SMN1-to-SMN2 gene conversion, impacts disease severity but lacks sufficient prognostic value. With newborn screening and SMN-targeted therapies, identifying genetic markers for disease progression and treatment response is critical. SMN2 DNA methylation has been proposed as such biomarker, but methodological limitations have hindered progress.

Material and Methods: We developed HapSMA, a polyploid phasing method for SMN locus analysis, enabling copy-specific assessment of genetic variation and DNA methylation using long-read sequencing. We applied HapSMA on publicly available nanopore sequencing data of 29 healthy controls and performed long-read, targeted nanopore sequencing of the *SMN* locus of 31 patients with SMA. Differential DNA methylation was validated in 365 SMA patients using targeted bisulfite sequencing.

Results: In healthy controls, we identified SMN1- and SMN2-specific SNVs as potential gene conversion markers. Broad phasing including the *NAIP* gene improved our view of *SMN* locus variation. Genetic variation in *SMN2* haplotypes was larger in SMA patients. 42% of *SMN2* haplotypes of SMA patients showed varying *SMN1* to *SMN2* gene conversion breakpoints, serving as direct evidence of gene conversion as a common genetic characteristic in SMA. However, SMN2 DNA methylation showed no correlation with disease severity or treatment response, excluding blood methylation patterns as predictive biomarkers.

Conclusion: Our findings highlight the need for methodological advancements and patient-based analyses to resolve complex genetic loci and address critical clinical challenges in SMA.

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P12.007.C Recessive variants in *DARS2* gene as a novel cause of axonal Charcot-Marie-Tooth disease

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Background: Pathogenic variants in the mitochondrial aspartyl-tRNA synthetase 2 (*DARS2*) gene are known to cause leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL). Here we report five individuals from three unrelated families with recessively inherited variants in *DARS2* presenting as isolated axonal Charcot-Marie-Tooth (CMT) phenotype.

Material and Methods: We retrospectively collected clinical information of all individuals and performed functional studies on three affected persons.

Results: All individuals exhibited progressive distal muscle weakness with onset in childhood and absent lower limb deep tendon reflexes. Electrophysiological studies supported an axonal motor and sensory neuropathy. Two pairs of adult siblings had normal cognitive functions, whereas the fifth patient presented with speech delay, moderate intellectual disability, and generalized epilepsy with onset at age eleven. Brain MRI scans showed no white matter abnormalities or elevated lactate in any of the patients. Whole exome sequencing identified compound heterozygous variants in *DARS2* gene (NM_018122.5) across all families (c.713C>T; p.Ser238Phe and c.1006C>T; p.Arg336Cys in family 1; c.74del; p.Ile25Thrfs*38 and c.713C>T; p.Ser238Phe in family 2; and c.492+2T>C and c.1508C>T p.Pro503Leu in family 3). Functional analysis demonstrated reduced DARS2 protein activity (25% for the missense c.1508C>T variant) and altered mitochondrial localization, accompanied by disrupted mitochondrial network morphology. Muscle biopsy from patient five showed marked fibrosis, fatty tissue infiltration, numerous groups of atrophic muscle fibers, and uniformity of type 2 fibers.

Conclusion: We describe a new phenotype of mostly pure axonal CMT in five individuals from three unrelated families, associated with recessively inherited variants in the *DARS2* gene.

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P12.008.D Identification of differential expressed serum microRNAs as potential biomarkers for myotonic dystrophy type 1 and facioscapulohumeral muscular dystrophy

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Background: Myotonic dystrophy type 1 (DM1) and facioscapulohumeral muscular dystrophy (FSHD) are common adult-onset muscular dystrophies characterized by progressive muscle weakness. Given their prevalence, reliable and easily accessible biomarkers are needed for accurate diagnosis. Recent studies suggest disease-specific microRNAs (miRNAs) expression patterns, which may provide insights into disease pathogenesis and novel diagnostic strategies. This study aims to identify potential miRNA biomarkers through comparative expression analysis in healthy controls, DM1, and FSHD groups.

Material and Methods: Small RNA was extracted from serum using the miRNeasy Serum/Plasma Kit, and library were prepared and sequencing was performed on the Illumina Nextseq2000 platform with 100-bp paired-end reads. Differential expression analysis was conducted using the CLC Genomics Workbench 25.0, modeling read counts with a Negative Binominal distribution. Differentially expressed miRNAs were identified based on an False Discovery Rate (FDR)-adjusted p-value (<0.05) and an absolute | log2(fold change)|≥1.

Results: A total of 93 serum samples, including 22 controls, 34 DM1, and 37 FSHD1 patients, were analyzed for differential mRNA expression. In the DM1 group, 30 miRNAs were significantly differentially expressed, with hsa-miR-1306-5p, hsa-miR-483-3p, and hsa-miR-885-5p demonstrated the most notable changes (FDR < 0.05) after applying a Counts Per Million (CPM) threshold of >10. In the FSHD1 group, 16 miRNAs initially met the criteria for differential expression, with 13 retained after filtering. While none reached FDR, hsa-miR-4307, hsa-miR-206, and hsa-miR-133a-3p exhibited substantial upregulation in FSHD patients compared to controls.

Conclusion: This study identifies showed distinct serum miRNA expression profiles in DM1 and FSHD1 and identified hsa-miR-1306-5p, hsa-miR-483-3p, and hsa-miR-885-5p as potential biomarkers for DM1.

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P12.009.A Limitations in facioscapulohumeral muscular dystrophy (FSHD) diagnostics and the potential of nanopore long-read sequencing

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Background: Despite its often characteristic clinical phenotype, diagnosing FSHD remains challenging, with approximately 5% of cases lacking a molecular genetic diagnosis. Distinct methylation profiles have been identified as key biomarkers reflecting the complex genetic basis of FSHD, offering improvements in genetic diagnostics.

Material and Methods: We analysed the methylation profiles of over 150 patients using a bisulfite assay and identified 12 cases that would have received a false-positive or false-negative diagnosis using standard diagnostic methods. To achieve a comprehensive characterization of the genetic and epigenetic parameters, we further examined these 12 patients, along with 8

additional FSHD2 cases, using Nanopore long-read sequencing. This method enabled simultaneous analysis of allelic haplotypes, repeat units (RUs), methylation profiles, single nucleotide variants, structural variations and hybrid alleles.

Results: We identified 10 patients who would have received a false-negative diagnosis using standard diagnostics. These individuals carried either a grey zone allele of 11 RUs or no detectable variant in known epigenetic suppressor genes, despite presenting with global hypomethylation associated with FSHD2. One patient exhibited characteristics of both FSHD1 and FSHD2, while another patient was excluded from an FSHD diagnosis due to the presence of a non-permissive hybrid allele.

Conclusion: Our results reinforce the value of methylation profiling as the most precise biomarker for FSHD diagnostics and suggests the existence of additional unknown factors acting as methylation modifiers. Integrating genetic with epigenetic data using long-read sequencing improves diagnostic accuracy and broadens our understanding of the underlying pathomechanisms of FSHD.

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P12.010.B Novel functional insights into TTNmissense variants

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Background: Variants in the TTN gene have been identified in over 25% of all cases of dilated cardiomyopathy, and biallelic TTN truncating variants (TTNtv) are among the most prevalent causes of myopathies. Approximately 1 in 20 individuals carries a rare missense TTN variant (MAF <1%), and the TTN protein has over 600,000 possible amino acid alterations. Nearly all these are currently classified as variants of uncertain significance.

Material and Methods: Patients' clinical and genetic data were collected from various international hospitals. The study includes preliminary in-vitro experimental evidence on selected missense variants. We cloned the sequences encoding wild type (WT) and variant titin domains into a vector for eukaryotic cell expression with an N-terminal GFP-tag. Transfected COS-7 cells were cultured for 2 days, fixed, and then analyzed.

Results: In an overall analysis, we calculated that 33% of all variants absent in GnomAD and 64% of all possible proline substitutions are predicted likely pathogenic by AlphaMissense (score > 0.5). We then identified 15 myopathy patients with a TTNtv in trans with a missense variant. For each missense variant, we assessed pathogenicity score with AlphaMissense, minor allele frequency (MAF) from GnomAD, and the nature of the amino acid substitution. In selected cases of proline substitutions located in beta-strands, experimental results showed an aggregated appearance of the mutated domain.

Conclusion: This study identified likely pathogenic TTN missense variants, highlighting the destabilizing effect of proline substitutions in beta-strand domains. It also offered initial insights into genotype-phenotype correlations in these underinvestigated forms of recessive titinopathies.

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P12.011.C From genomes to phenomes: Discovering *DMPK* repeat expansions in Genomics England for future PheWAS

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Background: Myotonic Dystrophy Type 1 (DM1) is an autosomal dominant disorder caused by CTG repeat expansions in the *DMPK* gene. Typically, healthy individuals have 5–37 repeats; those with premutation alleles (38–49 repeats) may be asymptomatic, carry the risk of intergenerational expansion. Pathogenic expansions (>50 repeats) lead to DM1's multisystemic symptoms. Despite well-established clinical features, the population-level impact of *DMPK* expansions, particularly across different genetic ancestries, remains underexplored. Leveraging the Genomics England (GEL) data, we aim to (1) identify and classify *DMPK* expansions, (2) analyse repeat length distributions across ancestries, and (3) establish a genotype-phenotype database linking individuals to Human Phenotype Ontology (HPO) terms. Thus, laying the groundwork for a future phenome-wide association study (PheWAS) utilising this data, representing the first application of an HPO-based PheWAS in a DM1 context.

Material and Methods: We analysed 80,110 GEL whole-genome sequencing samples for *DMPK* expansions using Expansion Hunter, categorising participants into normal (<37 repeats), premutation (38–49 repeats), and pathogenic (>50 repeats) cohorts. Statistical analyses assessed ancestry-based patterns in repeat distributions.

Results: Among 80,110 genomes, we identified 78 participants with pathogenic expansions, 183 with premutation alleles, and 79,849 with normal repeat lengths. Significant ancestry-based differences were observed in comparisons between pathogenic

vs. normal cohorts ($p = 0.038$) and normal vs. premutation cohorts ($p = 0.003$), $p < 0.05$.

Conclusion: This study identified significant ancestry-related differences in *DMPK* expansions with a robust HPO-linked database being developed to support the planned PheWAS. Thus, paving the way for the first HPO-based PheWAS investigating pleiotropic effects of *DMPK* expansions, broadening our understanding of DM1 across populations.

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P12.012.D Structural variation in nebulin and its impact on phenotype and inheritance: establishing a dominant distal phenotype caused by large deletions

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Background: Structural variants in the nebulin gene (*NEB*) are an established cause of recessive nemaline myopathies and related neuromuscular disorders. We present data from 35 families with muscle disorders caused by *NEB* intragenic structural variants, including 18 previously unpublished cases.

Material and Methods: Our cohort consists of 35 families; of these, 12 families have dominantly inherited *NEB*-related distal myopathy phenotypes (10 unpublished) caused by large deletions, and 23 families have recessively inherited myopathies caused by pathogenic gains of the *NEB* triplicate region ($n = 12$, 5 unpublished) or other structural variants ($n = 11$, 3 unpublished) either in a homozygous or compound heterozygous state. Variants were detected using exome or genome sequencing, Droplet Digital PCR, or custom Comparative Genomic Hybridization arrays. Sanger sequencing was used to validate breakpoints in applicable cases.

Results: In families affected by dominant distal myopathy phenotypes, we identified eight unique *NEB* deletions encompassing 51 to 97 exons in heterozygous ($n = 10$) or mosaic ($n = 2$) state. Among the recessive cases, no correlation was observed between the type of variant and the disease severity.

Conclusion: We establish a statistically significant association between large *NEB* deletions and a dominant form of *NEB*-related distal myopathy ($p < 0.001$). Additionally, we provide the most comprehensive overview to date of the spectra of structural variants in *NEB*.

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P12.013.A Mutational Spectrum of Hereditary transthyretin-mediated amyloidosis-hATTR in Italian population: results from the Genilam Project

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Background: Hereditary transthyretin-mediated amyloidosis-hATTR, caused by heterozygous variant in the transthyretin (TTR) gene, is a potentially life-threatening condition for which targeted therapies dramatically changed the prognosis. Highly variable clinical presentation makes hATTR a diagnostic challenge because of subclinical and non-specific presentation. Since 2019, Italian clinical centres were offered the possibility of TTR testing in the project Genilam

Material and Methods: We retrospectively evaluated results for samples analysed between June 2019 and December 2023. DNA was obtained by buccal swab; TTR testing was performed by Sanger sequencing of exons 2, 3 and 4. TAT: 15 working days.

Results: A total of 7536 samples from >200 specialized medical centres were tested. A total of 372 patients (372/7536; 4.93%) tested positive, including 3 compound heterozygous and 2 homozygous. The most common amyloidogenic variants were Phe84Leu (130/372; 32.3%), Val50Met (80/372; 19.8%), Ile88Leu (68/372; 16.9%), Val142Ile (40/372; 9.9%), Glu109Gln (31/372; 7.7%). Non-amyloidogenic variants were also identified (His110Asn: 17/372, 4.5%; Arg124Cys: 3/372, 0.80%; Ala129Thr: 1/372, 0.26%; Thr139Met: 7/372, 1.88%). Most patients identified as positive were male (220/372; 59.1%). Number of positive results for each region was evaluated according to both location of referring center (RC) and place of birth (PoB). Three regions showed the highest incidence for both parameters: Sicilia (25.5% RC; 26.9% PoB), Lazio (24.5% RC, 18.5% PoB), and Puglia (10.8% RC, 13.2% PoB).

Conclusion: Genilam project allowed rapid diagnosis improving management timely identifying affected individual. The project will also redefine the spectrum of variants in Italy allowing for improvement of genotype phenotype correlation

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P12.014.B Detecting distant relatedness between ALS patients to facilitate gene discovery

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Background: Amyotrophic lateral sclerosis (ALS) is fatal neurodegenerative disease. The search for underlying pathogenic variants is ongoing. The construction of so-called superpedigrees, extended families with multiple distantly related ALS patients, is highly valuable for the detection of low-frequency genetic variants with large effect but moderate penetrance. Here, we explore bioinformatic pipelines that detect distant relatedness between individuals from genetic data.

Materials and Methods: We applied the methods (TRIBES[1], DRUID[2] and GRAPE[3]) to a dataset of 3,842 Dutch ALS cases and controls from Project MinE, and compared them based on speed, scalability, usability and ability to identify clusters of related individuals.

Results: GRAPE was the most computationally efficient workflow, whereas TRIBES was the most sensitive. All methods detected significantly more relationships (up to the sixth degree) between individuals with the *C9orf72* repeat expansion than would be expected by chance. Furthermore, network analyses revealed higher clustering of ALS patients compared to controls, validating the accuracy of the approach. We discovered three extended pedigrees: 1) linked familial ALS cases with an unresolved pathogenic variant, partially previously found by genealogy; 2) interconnected sporadic ALS cases from an isolated village in the Netherlands; 3) a *C9orf72* positive family with two *C9orf72* positive sporadic ALS cases not previously linked to the family. Unresolved pedigrees are prime targets for haplotype and linkage analysis.

Conclusion: Our findings suggest that the reconstruction of extended superpedigrees through the application of these methods, validated by traditional genealogy, is a promising approach for identifying new ALS risk genes.

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P12.015.C Rapid *SMN1* and *SMN2* copy number analysis by digital PCR (dPCR) in 6 hours to facilitate neonatal SMA therapy treatment

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Background: Spinal Muscular Atrophy (SMA) is a neuromuscular disorder that affects ~1 in 100,000 live births with ~94% having a homozygous deletion of *SMN1* exon 7. Molecular diagnosis is complicated by the presence of a pseudogene, *SMN2*. Recently, treatments including gene therapy have become available which can improve these patients' outcome but as neurodegeneration occurs at ~34 weeks *in utero*, rapid diagnosis is critical. To facilitate this, we have developed and validated two dPCR assays.

Material and Methods: Two multiplex dPCR assays (A & B) were developed using the same primers and probes for *SMN1* and *SMN2* exon 7 and controls, *RPPH1* and *TERT;* but using different fluorescent dyes for the probes. 71 DNA samples extracted from a variety of tissues and with 0–4 copies of *SMN1* and 0–5 copies of *SMN2*, determined by MLPA, were tested.

Results: 69 of 71 samples with dPCR gave the same genotypes as MLPA analysis. The two discordant samples had a MLPA *SMN2* copy number call of 4/5 and 5, but dPCR (A & B) *SMN2* calls of 5 and 4, respectively. Further testing of these samples are underway. 34 samples were retested using both assays generating the same genotypes, demonstrating the assay's reproducibility. The same genotypes were obtained using final DNA concentrations from 0.3 to 2 ng/µL, defining the limit of detection.

Conclusion: We have designed and validated two dPCR assays for the rapid and accurate diagnosis of SMA patients, enabling them to have earlier access to treatment, significantly affecting their quality of life.

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P12.016.D Expanding the *FDXR*-associated disease phenotype and defining the underlining disease mechanisms

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Background: Ferredoxin reductase (*FDXR*) is a mitochondrial flavoprotein crucial for iron-sulfur cluster (ISC) and heme biosynthesis, initiating the electron transport chain (ETC) by transferring electrons from NADPH to cytochrome P450 system. Mutations in *FDXR* cause a rare recessive neurological disorder, characterized by visual and hearing impairment, with onset in first or second decade of life. Disease severity varies, with some cases exhibiting debilitating neurological symptoms. However, the mechanisms underlying the clinical manifestations remain unclear. Here, we investigate clinical, molecular and biochemical data of two patients with novel *FDXR* mutations.

Material and Methods: Patient-derived fibroblasts were used for molecular and functional analysis.

Results: Patient 1 (P.1) presents severe retinal degeneration, acute pancerebellar syndrome, and red-cone dystrophy with no optic atrophy. Patient 2 (P.2) developed progressive visual disturbances with optic atrophy, nystagmus, and mild sensorineural deafness at 16 years. Sanger sequencing revealed an unreported homozygous variant, c.1472G>A (p.R491Q), in P.1 and two compound heterozygous variants, c.1231G>A (p.D411N) and c.1A>G (p.M1V), in P.2. Mitochondrial copy number was increased in *FDXR* patients' fibroblast cell lines. Both P.1 and P.2 showed reduced FDXR steady-state protein levels. In P.2, levels of ETC ISC-containing subunits as Ndufs1 and SDHB were decreased, while oxidative stress-related proteins, Aconitase-2 and SOD2, were dysregulated in both patients. Additionally, P.2 exhibited significant impairment in oxygen consumption rate, with reduced basal and maximal respiration.

Conclusion: Our findings confirm the presence of deleterious oxidative stress in two patients with novel *FDXR* mutations. Future studies will elucidate the pathogenic mechanisms and explore potential therapeutic strategies.

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P12.018.B Aminoacyl-tRNA synthetase defects in Charcot-Marie-Tooth (CMT) disease

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Background: Charcot-Marie-Tooth (CMT) disease, affecting 1 in 2500 individuals globally, is characterized by progressive peripheral neuropathy. CMT presents significant phenotypic and genetic heterogeneity, with over 150 implicated genes including seven aminoacyl-tRNA synthetases (aaRS) associated with autosomal dominant CMT type 2. These enzymes are essential for protein synthesis by charging tRNA with cognate amino acids. Advancements in genome sequencing have uncovered numerous aaRS variants, often classified as variants of uncertain significance (VUS). We investigated aaRS variants to characterize their pathogenicity and potential response to amino acid supplementation.

Material and Methods: We studied patients with peripheral polyneuropathy and aaRS variants, including two patients with HARS1 variants: p.Y138H (patient 1) and p.L400F (patient 2), initially classified as VUS. Humanized yeast models and biochemical assays were used to assess variant pathogenicity and amino acid supplementation efficacy.

Results: Both patients presented with peripheral polyneuropathy and non-classical symptoms including burning sensations and weakness in the neck, arms, and legs, with exhaustion exacerbated by febrile episodes and exposure to high ambient temperatures. Humanized HARS1 yeast models and biochemical analyses revealed deficient protein dimerization and growth defects, supporting their pathogenicity. Histidine supplementation showed mild but significant improvement for p.Y138H but reduced growth for p.L400F; alternative therapeutic approaches are currently being evaluated for this variant.

Conclusion: Understanding the molecular mechanisms of aaRS variants in CMT is crucial for characterizing VUS pathogenicity and developing targeted therapies. As demonstrated with these HARS1 variants, different alleles may require different therapeutic interventions for advancing personalized treatments.

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P12.019.C Family-based gene discoveries in ALS: comprehensive literature review and analysis of Dutch familial ALS cohort

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease showing marked locus heterogeneity in familial cases. Family-based studies have been crucial in identifying ALS-associated genes.[1] The recent approval of Tofersen as a treatment for SOD1-linked ALS underscores the significance of elucidating the complete genetic architecture of familial ALS (FALS).[2,3]

Methods: We compiled ALS-associated genes from family-based studies and screened variants in WGS data from FALS cases in our national biobank up to March 2024. Variants were annotated using ANNOVAR, incorporating gnomAD, ClinVar, and refGene data. We excluded non-coding and synonymous variants, and those with MAF $>1.0 \times 10^{-4}$ in Non-Finnish Europeans. Using adapted ACMG guidelines[4–6], we classified variants and assessed pedigrees to identify those with a causal variant.

Results: We analysed 111 pedigrees, including 140 cases, predominantly with ALS (89.3%). Other diagnoses included primary lateral sclerosis, progressive muscular atrophy, and segmental spinal muscular atrophy. We detected C9orf72 repeat expansions and explored SNVs in 38 genes across the cohort. 63 pedigrees (57%) were resolved, with 52 having C9orf72 as the sole causal variant. Other causal genes included TARDBP (5/111), CHCHD10 (1/111), FUS (1/111), SOD1 (1/111), and TBK1 (1/111). Two pedigrees had multiple causal variants: C9orf72 and NEK1 in one, C9orf72 and TARDBP in the other. Among the unresolved pedigrees, 44 had no identifiable causal variant, while 4 contained VUS.

Conclusion: Most pedigrees were resolved, while a causal locus remains unidentified in over 40%. WGS of extended pedigrees where familial and sporadic branches may be connected could help uncover new causative loci.

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P12.020.D Prevalence of Late-Onset Pompe Disease-causing variants in the UK Biobank

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Background: Pompe disease (PD), is a disorder due to biallelic pathogenic variants in *GAA*, resulting in the enzymatic deficiency of alpha-1,4-glucosidase. Two clinical forms are recognized: early- (EOPD) and late-onset (LOPD). LOPD manifestation is heterogenous with variable degrees of severity and progression rate. Some LOPD patients remain asymptomatic for decades. The estimate of incidence is around 1/40,000 overall (3/4 as LOPD and 1/4 as EOPD). We aimed to determine the prevalence of LOPD disease in UK Biobank.

Material and Methods: We sought GAA gene variants in exome sequencing data from 470 000 individuals aged 40 years or older). We focused on variants previously established as pathogenetic in the GAA variant database at Erasmus MC in Rotterdam and on Class 4 or 5 variants (ACMG guidelines).

Results: The common variant IVS1-32-13T>G (IVS1) displayed a carrier frequency of 1.3%. 17 subjects were found homozygous for IVS1. 74 subjects presented IVS1 and a second pathogenetic defect with (n = 15) or without (n = 59) conclusive demonstration of pathogenicity: less than 10% of them presented clinical symptoms compatible with LOPD. Additional 8 subjects harbored two (non IVS1) coding variants, reported with a mild-to-moderate pathogenic burden. If biallelic inheritance was proven, the genetic prevalence of LOPD-causing variants in UK Biobank is 1/4700.

Conclusion: The genetic prevalence of LOPD likely exceeds traditional and recent (1/18000, newborn screening programs) estimates. Genotypes observed are likely not associated with a severe course of the disease. Nevertheless, given the existence of therapeutic options, an in-depth follow up of these subjects is advised.

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P12.021.A Expanding the genetic and phenotypic spectrum of *RYR1*-related diseases

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Background: *RYR1*-related diseases are a group of rare genetic disorders caused by variants in *RYR1*, which encodes the ryanodine receptor 1 (RyR1). These manifest as a spectrum of neuromuscular conditions, including well-known phenotypes such as central core disease, multiminicore disease, and malignant hyperthermia susceptibility, as well as viral infection- and exercise-induced hyperCKemia. The study aims to explore the genetic and clinical features of *RYR1*-related diseases in a cohort of affected individuals.

Material and Methods: Seventeen patients (ages 0–69 years) from 16 unrelated families were referred for Whole exome sequencing (WES) due to variable clinical presentations, ranging from asymptomatic hyperCKemia and recurrent rhabdomyolysis episodes to severe muscle weakness. Clinical, genetic, and histopathological data were assessed to allow phenotype-genotype correlations.

Results: Twenty-four, of which 7 novel, distinct pathogenic/likely pathogenic *RYR1* variants were identified, most of them missense, following both autosomal dominant (AD) and autosomal recessive (AR) inheritance. In 3 out of 16 patients with congenital myopathy the previously associated with malignant hyperthermia, c.[4711A>G;10097G>A;11798A>G] haplotype was detected in compound heterozygosity. In five patients, muscle biopsies were available and revealed "Central core" pathology (n = 2), centronuclear pathology (n = 1), congenital fiber-type disproportion (CFTD) (n = 1) and predominance of type I muscle fibers (n = 1) which were concordant with molecular diagnosis.

Conclusion: Identification of seven novel pathogenic *RYR1* variants, further expands the genetic and phenotypic spectrum of *RYR1*-related diseases highlighting the broad genetic heterogeneity and the association between specific variants and clinical phenotypes.

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P12.023.C Parallel analysis of repeat expansions: an updated Clin-CATS workflow for nanopore R10 flow cells

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Background: Hereditary ataxias caused by short tandem repeat (STR) expansions are difficult to diagnose with conventional PCR and Southern blot methods, which fail to detect complex expansions, repeat interruptions, and methylation. Long-read sequencing, particularly Oxford Nanopore Technologies (ONT) platforms, provides a viable alternative but requires workflow adaptation.

Material and Methods: We present an updated Clinical Nanopore Cas9-Targeted Sequencing (Clin-CATS) workflow optimized for R10 flow cells. The workflow integrates the ONT wf-human-variation pipeline with Straglr for repeat expansion analysis. The number of analyzed genes was expanded from 10 to 27, introducing new panels for neurological disorders. Validation was performed using Coriell reference and clinical samples, and multiplexing, use of Flongle flowcells, and single-gene targeting were assessed.

Results: Protocol modifications achieved an average coverage of 222× across all targets, compared to 439× with R9 flowcells. Mean read quality improved from 15.3 (R9) to 16.5 (R10), reaching 17.5 in Coriell samples. Multiplexed sequencing with 2, 4, and 6 samples yielded only 3–5× coverage and Flongle flowcells 3.2×, failing to meet the 50× diagnostic threshold. Single-gene targeting performed comparably to full-panel sequencing. Integration of Straglr improves repeat expansion analysis and ensures compatibility with all sequencing chemistries as it relies on basecalled data.

Conclusion: The updated Clin-CATS workflow improves diagnostic utility for STR-associated disorders. Although coverage is reduced compared to R9, the updated workflow meets the required diagnostic thresholds and improves repeat expansion detection overall.

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P12.024.D Characterization of clinical and genetic features of *SIGMAR1*-related disorder

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Background: Bi-allelic variants in Sigma Nonopioid Intracellular Receptor 1 (*SIGMAR1*) have been associated with distal hereditary motor neuropathy (dHMN) and juvenile amyotrophic lateral sclerosis. Current understanding is based on case reports and small series. We evaluated genotype-phenotype correlations in individuals with biallelic *SIGMAR1* variants.

Material and Methods: Through exome sequencing and international data sharing, we identified 16 affected individuals from 15 families. Phenotypic and genetic data from these cases and available data from previously reported cases were analyzed.

Results: Seventy-seven patients from 37 families were included in the study. Seven novel and eight recurrent *SIGMAR1* variants were identified, including nine missense, four frameshift, and two nonsense changes. Most patients exhibited a uniform phenotype of symmetrical distal weakness, with varying degrees of pyramidal features. Exceptions included three individuals with asymmetrical muscle weakness or atrophy. The disease manifested in the first or early second decade of life, except in three patients harboring different biallelic variants. Lower extremity symptoms preceded upper extremity involvement by an average of 4.8 ± 4.1 years. The mean age was 28.6 ± 18.5 years at the last clinical assessment. Patients remained ambulatory (with or without support) for a mean disease duration of 15.6 ± 11.3 years. Distal weakness were universally observed, while proximal weakness occurred in 7 out of 37 patients with available clinical data. Notably, bulbar features, respiratory dysfunction, and sensory signs were absent in all patients.

Conclusion: This study expands the genotypic spectrum and characterizes the phenotypic presentation of *SIGMAR1*-related dHMN, highlighting its consistent clinical features.

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P12.025.A Latin-SEQ: a collaborative network to provide genetic diagnosis to patients with neuromuscular diseases in Latin-America

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NGS has revolutionized the diagnostic process of patients with neuromuscular diseases (NMDs). However, access to these technologies in less developed regions, such as Latin American countries is highly limited, leaving patient without a diagnosis and therefore no proper counselling, management, or access to therapies or trials. We created a consortium of specialised centers with the objective of providing undiagnosed NMD patients a much-needed opportunity for genetic testing. We will also study disease prevalence, region-specific genotype-phenotype correlation, founder mutations, and novel causative genes.

Results: The Latin-SEQ consortium was stablished and currently comprises >60 centers from 18 countries in Latin America. Biomaterial from NMD patients and their family members is recruited locally and processed and biobanked in Newcastle, currently totally 1230 samples. Detailed phenotypic data is collected in an HPO-based clinical database. Proband-only exome sequencing is performed and the data analysed through the Genome Phenome Analysis Platform. Genetic variants are classified following ACMG guidelines, and individual cases discussed in patient-centered MDT meetings to reach an expert-level consensus. A total of 350 exomes have been so far analysed with a diagnostic yield of 38%. A novel candidate gene has already been identified in the Latin-SEQ cohort. Analysis continues.

Conclusions: After numerous administrative and logistic challenges, the Latin-SEQ consortium continues to grow. The phenotype-driven screening pipeline allowed a diagnosis of ~40% of cases, eight of whom will start life-changing treatment soon. The consortium has also created an international framework for further collaborative research in the region to obtain much needed genetic and epidemiological data.

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P12.026.B Abstract ESHG 2025 A multi-omics approach to study monozygotic twins discordant for amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by motor neuron degeneration. The majority of ALS cases are sporadic, while 10% are familial: part of heritability remains unexplained, suggesting the involvement of other genetic and epigenetic factors.

Material and Methods: We used a multi-omics approach in a pair of Italian monozygotic twins discordant for ALS, integrating genome-wide DNA methylation, bulk transcriptomics and whole exome sequencing. Furthermore, we performed a systematic review of the literature on monozygotic twins and sALS, to highlight possible general disease mechanisms.

Results: No pathogenetic variants were observed among twins. Conversely, transcriptomic analysis identified 42 differentially expressed genes (p.adj < 0.1; |log2FC|>1). Functional analyses on DEGs (Metascape) revealed the involvement of adaptive and innate immune system pathways. Moreover, epigenetic analysis identified 250 differentially methylated probes (p.adj < 0.05; $|\Delta\beta| < 0.06$) and 17 regions (padj < 0.05), and enrichment analysis highlighted the involvement of brain related terms. A late integration of the three omics data revealed no shared gene. Notably, we analyzed for the first time the results of all literature studies on discordant ALS twins (45 twins from 6 studies) and of 9 studies on 7046 sALS and 3110 controls, and compared literature data to ours. We confirmed the involvement of immune system and brain related terms, suggesting shared pathogenetic mechanisms across ALS cases.

Conclusion: results obtained by applying a multi-omics approach to a small, well-characterized cohort were confirmed on a higher number of ALS subjects, providing pathological insights into ALS with a focus on neurodegenerative and immune-related processes.

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P12.027.C Duchenne muscular dystrophy and delandistrogene moxeparvovec gene therapy in children: a systematic review and meta-analysis

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Background: Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder caused by mutations in the *DMD* gene, leading to the absence of functional dystrophin. This results in muscle degeneration, loss of ambulation, respiratory failure, and reduced life expectancy. Current treatments, including corticosteroids and supportive care, offer limited long-term benefits. Delandistrogene Moxeparvovec is a promising gene therapy designed to restore dystrophin expression. This systematic review and meta-analysis assess its efficacy in ambulatory pediatric patients with DMD.

Methods: We searched Cochrane, PubMed, and Embase (January 2025) for RCTs and cohort studies evaluating Delandistrogene Moxeparvovec in ambulatory male children (≥4 to <8 years) with DMD. Primary outcomes included NSAA score changes, 10-meter Walk/Run (10 MWR), and Time To Rise (TTR). Study selection, data extraction, and quality assessment followed PRISMA guidelines. Statistical analyses were performed using R software (Version 4.2.3). PROSPERO registration: CRD42025635605.

Results: We included 276 patients from three studies (two RCTs). Follow-up ranged from 48 to 52 weeks. All had confirmed *DMD* mutations (exons 18–79) and received corticosteroids for ≥12 weeks. Delandistrogene Moxeparvovec (1.33×10^{14} vg/kg) was given to 104 patients; 174 were in placebo or external control groups. TTR improved significantly (MD: −0.85s, 95% CI: −1.23 to −0.47, P < 0.01). NSAA (MD: 1.52, P = 0.06) and 10 MWR (MD: −0.27s, P = 0.57) showed no statistical significance.

Conclusion: Delandistrogene Moxeparvovec may improve functional outcomes in DMD patients aged ≥4 to <8 years. Therefore, Delandistrogene Moxeparvovec may be a therapeutic

option for patients with DMD. However, further RCTs with longer follow-ups are needed to confirm long-term efficacy.

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P12.028.D Assessment of an explainable AI model for variant classification in neuromuscular disorders

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Background: Neuromuscular disorders (NMDs) are a diverse group of inherited conditions caused by genetic mutations, many of which remain classified as Variants of Uncertain Significance (VUS). This classification creates significant challenges in providing patients with a definitive genetic diagnosis. Reclassifying these variants using up-to-date evidence is essential to improve diagnostic outcomes. In recent years, artificial intelligence (AI) has emerged as a promising approach for tackling this challenge. Here, we evaluate the performance of an AI-based meta-predictor designed to classify novel NMD variants with high accuracy.

Material and Methods: The meta-predictor [1] was trained on ClinVar 2023 data. The variants selected for evaluation have been described in a multi-national study [2], consisting of 33 variants with clear clinical significance for testing and 34 VUS to explore potential reclassifications. Notably, 95% of these variants were absent from ClinVar, making this a challenging benchmark for assessing the tool's accuracy. ACMG guideline-based classifications done by experts have been used as the ground truth for validation [2].

Results: The AI model accurately classified 90% of the pathogenic variants in the test dataset. It also provided reclassifications for 86% of VUS, suggesting that many of these variants may eventually achieve clinical significance as more evidence becomes available.

Conclusion: This study demonstrates the effectiveness of an AI-based meta-predictor for classifying different types of variants associated with NMDs. By addressing the challenges of VUS, our tool shows promise for improving genetic diagnostics and supporting clinical decision-making. Continued validation will be important to ensure its broader application in real-world settings.

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P12.029.A Using patient-derived iPSC lines for calpainopathy research and therapy development

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Background: Calpainopathy is a progressive autosomal recessive limb-girdle muscular dystrophy (LGMDR1) caused by calpain 3 (*CAPN3*) gene variants. We previously identified a hypomorphic intronic variant, c.1746-20C>G, common in Latvia (MAF 0.24) and other Eastern European countries, classified as having conflicting interpretations of pathogenicity. We showed it causes aberrant splicing, likely due to a disrupted branch point, and contributes to disease phenotype when in a compound heterozygous state with another pathogenic variant in the gene.

Materials and Methods: Fibroblasts from patient skin biopsies were transduced with Sendai virus to generate iPSC clones. Colonies were screened for virus clearance, and pluripotency was assessed via marker expression and trilineage differentiation (immunofluorescence, TaqMan hPSC scorecard). Genetic stability was evaluated using CMA assay and G-banded karyotyping. STR profiling was conducted on both iPSCs and the original fibroblasts. iPSCs were differentiated using the appropriate differentiation media and validated through marker expression.

Results: Three patient-specific iPSC lines were established, exhibiting PSC-like morphology and expressing Oct-4, Nanog, Sox2, SSEA-4, and Tra-1-60. Trilineage differentiation potential was confirmed, and STR analysis verified the identity of iPSCs. All lines showed genetic stability at p.11 and were successfully differentiated into myoblasts and myotubes. The cell lines are registered in the hPSCreg registry.

Conclusion: These cell lines provide a valuable in vitro model to study calpainopathy-affected muscles, offering an animal-free platform for future investigations of disease mechanisms and therapeutic strategies, including muscle-on-chip approaches.

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P12.031.C A Machine Learning approach to investigate digenic inheritance in Amyotrophic Lateral Sclerosis using Whole Genome Data

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Background: In 85% of ALS (Amyotrophic Lateral Sclerosis) cases the genetic cause is unknown. Oligogenic/digenic inheritance can explain the ALS missing heritability since 1–13% of cases carry two pathogenic variants in ALS genes.

Aim: To investigate the role of oligogenic/digenic inheritance in ALS using Machine Learning (ML) tools, either with in-house developed pipeline or utilizing DIVAs tool (Engenome s.r.l.), on WGS data.

Methods: From a pilot cohort of WGS from 60 patients, we used DIVAs to predict digenic gene pairs through an unbiased genome-wide investigation, assigning them a pathogenicity score ranging from 0 to 1. Moreover, we developed a semi-automatic pipeline for digenic data analysis with VarCoPP-HOP software through which we compared the DIVAs data of the pilot cohort and perform a discovery analysis prioritizing gene pairs from a larger WGS cohort of 4,090 ALS patients and 775 controls.

Results: In the pilot study using DIVAs we prioritized digenic combinations with a score higher than 0.6 and predicted to be Variants of Unknown Significance (VUS), Likely Pathogenic (LP) or Pathogenic (P). As an example, we found enriched heterozygous missense LP variants in *SPG7*, causative of autosomal recessive Spastic Paraplegia 7, paired with VUS in ALS genes, namely *TBK1* and *SQSTM1* gene. These digenic combinations were priotitized either by DIVAs and VarCoPP-HOP tools. The discovery analysis with VarCoPP-HOP on the larger case-control WGS is ongoing.

Conclusions: We successfully implemented a workflow capable of selecting digenic pairs from WGS data whose demostration on the involvement in ALS pathogenesis requires replication in a large cohort.

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P12.032.D Familial case of Bethlem myopathy caused by the insertion of an ALU element in *COL6A2*

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Background: Bethlem myopathy (BM) is the mild end of the spectrum of diseases caused by molecular alterations in *COL6A1*, *COL6A2*, *COL6A3* or *COL12A1*. Here, we present a three-generation familial case of BM, where six affected members presented childhood onset of symptoms, slow progressive proximal muscle weakness of upper and lower limbs, joint contractures, difficulty walking, and a typical collagenopathy CT pattern.

Material and Methods: A proband's DNA sample was initially analysed by whole exome sequencing, but no variants were identified in the *COL6* and *COL12* genes. Exome data was re-analysed applying a pipeline for copy number and structural variants as part of the Solve-RD project. Candidate variants were segregated. Collagen6 immunostaining and RNAseq was performed on proband's fibroblasts.

Results: Re-analysis of exome data detected a large heterozygous insertion in exon 10 of *COL6A2*. It was characterised as a ~321 bp insertion between positions c.989_990 (hg19:chr21:47536717), 10 bp before the 3' end of exon 10. The presence of a target site duplication and the conserved A5TACA6 motif indicate that the inserted sequence stems from an ALU element. The insertion co-segregates with the presence of clinical symptoms, being absent in non-affected family members. RNAseq analysis identified an alternative transcript lacking exon 10, leading to an in-frame deletion. Proband's fibroblast showed a collagen6 immunostaining pattern similar to BM control.

Conclusion: We confirmed the clinical diagnosis of BM by the insertion of an ALU element in COL6A2. To our knowledge, this constitutes the first report of a COL6-related myopathy caused by an ALU mobile element.

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P12.033.A Unlocking the genetic puzzle of Hereditary Peripheral Neuropathies: The power of reverse phenotyping and advanced sequencing

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Background: Hereditary peripheral neuropathies (HPN) comprise a heterogeneous group of disorders that cause progressive degeneration of peripheral nerves. Genetic testing is the gold standard for diagnosis, although many families remain unresolved. The genetic homogeneity of the Sardinian population reduces the overall heterogeneity. However, it makes interpreting the results more difficult due to a high prevalence of variants of uncertain significance (VUS), which do not have a well-defined framework for classification.

Material and Methods: We performed WES on 50 unrelated Sardinian patients with a confirmed or suspected diagnosis of HPN.

Results: Following filtering and classification, WES identified significant bi-allelic variants in two distinct patients in the PXN and MFN2, respectively. Heterozygous variants in the MME and MPZ genes associated with Charcot-Marie-Tooth neuropathy were also detected. Notably, we observed heterozygous variants in voltage-gated sodium channel (VGSC) genes, including SCN9A, SCN1A, and SCN4A, suggesting their potential role in phenotype as well as pain modulation. In one family with negative WES results, we identified a bi-allelic AAGGG repeat expansion in the RFC1 gene in three affected siblings. In the remaining 21 families, we detected VUS in potentially causative genes, whose pathogenic relevance will need to be clarified through in silico analysis and/or functional studies.

Conclusion: A reverse phenotyping approach aids in reassessing variant significance based on emerging clinical features, refining atypical phenotypes and VUS classification. In Sardinia, overcoming diagnostic challenges necessitates advanced in silico analyses, cellular studies, comprehensive phenotyping, and integrated genomic techniques like long-read and RNA sequencing, ultimately enhancing diagnosis and personalised medicine.

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P12.034.B Comprehensive molecular characterization of eight families with FSHD2

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Background: Facioscapulohumeral muscular dystrophy type 2 (FSHD2) represents about 5% of all FSHD cases, highlighting its rarity and clinical significance. The complex digenic pathogenesis of this condition poses significant challenges for both research and diagnostics.

Material and Methods: The clinical presentation of the patients was evaluated through physical examination, including the use of the FSHD-CCEF. The lengths and haplotypes of D4Z4 4q alleles were determined using a qPCR-based approach. Pathogenic variants in chromatin modifier genes were identified through WES/WGS. DNA methylation was analyzed using bisulfite conversion assays with PCR targeting three loci (4qAS, 4qAL, and DUX4), followed by deep sequencing. RT-PCR analysis for splicing variants was performed on cDNA derived from PBMCs of heterozygous probands and healthy controls.

Results: From 357 individuals in the Russian FSHD Patient Registry, we identified 8 probands lacking a contracted permissive 4qA allele. Genetic analyses of these probands revealed various variants in the *SMCHD1* gene, including missense, nonsense, splicing, deep intronic variants, and one extended deletion. Comprehensive molecular analysis was performed on 6 probands and their available healthy and affected relatives (11 individuals). In one family, the diagnosis of FSHD2 was excluded based on the collective data. In three distinct families, the analysis unequivocally confirmed the diagnosis of FSHD2. Two families exhibited ambiguous methylation values, complicating the diagnosis of FSHD2. Certain analyses remain pending for two families (8 individuals).

Conclusion: In total, we studied 8 families by comprehensive FSHD diagnostic approach, revealing better understanding of the connections between molecular data, clinical presentation, and disease prognosis.

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P12.035.C Expanded Clinical and Genetic Characterization of Autosomal Recessive HMGCR-Related Limb-Girdle Muscular Dystrophy

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Background/Objectives: Autosomal recessive limb-girdle muscular dystrophy type R28 (LGMDR28; OMIM #620375) is a recently identified subtype of limb-girdle muscular dystrophy with 15 individuals from six unrelated families carrying biallelic variants in the *HMGCR* gene reported to date. Here, we describe 10 individuals from five unrelated Middle Eastern families diagnosed with LGMDR28, all carrying homozygous *HMGCR* variants.

Methods: Using genome and exome sequencing, we identified the *HMGCR* variants. The family members were screened by Sanger sequencing.

Results: Beyond the previously characterized clinical features of LGMDR28, we report congenital onset in two unrelated families and liver failure in three affected individuals. Because of the new disease association, *HMGCR* is rarely included in panel tests. Using genome and exome sequencing, we identified two novel homozygous missense variants, c.2519G>A p.(Arg840Gln) and c.1784G>A, p.(Arg595His); and a homozygous in-frame deletion (c.1522_1524del, p.(Ser508del)) that has been previously reported in a compound heterozygous state with a second HMGCR variant.

Conclusion: Our findings strengthen the evidence for the pathogenic role of *HMGCR* variants and expand the phenotypic spectrum of LGMDR28.

C.O.I. Disclosure:

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P12.036.D Next generation sequencing for the diagnosis of primary muscle disorders

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Background: Primary muscle disorders are clinically and genetically heterogeneous. The achievement of a reliable molecular diagnosis can improve clinical management, provides appropriate genetic counseling and testing of relatives, and enables potential therapeutic options. We reviewed the molecular findings obtained by using diagnostic next generation sequencing protocols at Dino Ferrari Center.

Material and Methods: In the last 5 years, 230 patients with the suspect of primary muscle disease underwent NGS-based molecular testing consisting of i) custom targeted gene panels (n = 84) or ii) clinical exome sequencing (n = 146) followed by diagnostic interpretation of variants in genes associated with matched clinical phenotypes.

Results: Disease-causing variants were identified in 82 patients (36%: definitive diagnosis). Furthermore, likely pathogenic variants and/or variants of uncertain significance were found in 34 additional probands (15%: probable diagnosis). Diagnostic yield of the two approaches was similar (50.0% versus 50.7%). This rate tends to increase in patients affected with muscular dystrophies (54%) and congenital myopathies (61%) while it falls to 33% in patients with a suspect of metabolic myopathy. The simultaneous analysis of two affected members from the same family facilitated the establishment of a diagnosis (88%). Muscle biopsy was available for 143 patients (47% presenting a positive genetic reports) and resulted crucial for the interpretation of uncertain results.

Conclusion: Clinical exome sequencing allowed the analysis of additional OMIM genes leading to unexpected diagnosis or the enlargement of the clinical spectrum of known disease-related genes. Whole exome sequencing contributed to identify 3 novel disease genes in the last two years.

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P12.037.A Genetic modifiers of age-of-onset in hereditary TTR amyloidosis: a genome-wide association study

Gustavo Maximiano Alves[1], *Elisa Vegezzi*[2], *Stefano Facchini*[3], *Ilaria Quartesan*[4], *R Mussinelli*[5], *Mario Nuvolone*[6], *Y Razvi*[7], *Valentine Perrain*[8], *Matteo Taglapietra*[9], *K Càrdenas-Soto*[10], *Christiana Christodoulou*[11], *Luca Leonardi*[12], *Stefano Tozza*[13], *Alessia Argiro*[14], *S Menao*[15], *Paola Mandich*[16], *F Cappelli*[14], *F Manganelli*[13], *Daniel Sanchez-Tejerina*[17], *Maria Posadas-Martinez*[18], *Adolfo Arregui*[19], *Ricardo Reisin*[20], *Yoshiki Sekijima*[21], *M Ueda*[22], *Solange Kapetanovic Garcia*[23], *Maria Sevilla*[24], *Katrin Hahn*[25], *Anna Mazzeo*[26], *Marcia Waddington-Cruz*[27], *Kleopa Kleopas*[28], *Ricardo Rojas-Garcia*[29], *Pietro Guaraldi*[30], *Alvaro Gragera*[31], *Marie Theaudin*[32], *Ute Hegenbart*[33], *John Berk*[34], *Marco Luigetti*[35], *Eugenia Cisneros-Barroso*[36], *Carlos Casas-Novas*[37], *Mary Reilly*[4], *Wilson Marques*[1], *Violaine Planté-Bordeneuve*[8], *Isabel Conceicao*[38], *JD Gillmore*[7], *Carolina Lemos*[39], *Teresa Coelho*[40], *Albena Todorova*[41], *Intissar Anan*[42], *Laura Obici*[5], *Andrea Cortese*[43]

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Background: TTR amyloidosis is caused by mutations in the TTR gene, leading to misfolded proteins forming toxic amyloid deposits in the peripheral nerves and heart. There is striking variability across countries and within single families on age-of-onset (AOO) and clinical features, which cannot be entirely explained by the specific gene variant. Therefore, a contribution of additional genetic factors has been hypothesized. This work aimed at identifying genetic modifiers of AOO by a genome-wide association (GWA) approach.

Material and Methods: DNA from ATTRv patients was been genotyped by Infinium TM Global Clinical Research Array-24v1.0 (Illumina, standard backbone of ~1,204,769 SNPs) customized with ~3,000 additional SNPs. Demographic and clinical variables were collected through a standardized form by all participating centres.

Results: 2441 ATTRv patients were enrolled from 38 centres, including 1604 symptomatic individuals. 910 carried a common Val50Met mutation, while 694 carried a different pathogenic variant in TTR gene. 64 percent of patients were males (1024/1604) with Caucasian ancestry (1283/1546, 83%). Median AOO was 56.5 ± 15.1 years, (range 18–87 years). 1403 DNA samples have been already genotyped and 1313 passed quality control (call rate 0.95). A preliminary analysis on 1000 samples did not disclose any GWA significant locus associated with variable AOO outside TTR locus itself, but additional analyses on the entire cohort are ongoing.

Conclusion: These are the baseline features of the largest ATTRv cohort, including different ethnicities, mutations and AOO. Ongoing GWA analyses will aim at identifying genetic modifiers of the onset and phenotype of these highly heterogeneous conditions.

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P12.038.B Insertion of a processed pseudogene deep into intron 14 of the CLCN1 gene as a cause of Becker's myotonia congenita

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Background: Non-dystrophic myotonias include several autosomal dominant or recessive skeletal muscle channelopathies. Myotonia congenita is the most common and is caused by pathogenic variants in the *CLCN1* gene. Pathogenic variants induce the hyperexcitability of skeletal muscle fibers and cause myotonia. Genomes evolve and acquire new functions using different mechanisms, one of these is retrotransposition. Processed pseudogenes are transposable elements derived from the retrotransposition of mRNAs that insert into the genome contributing to its plasticity.

Material and Methods: Genetic diagnosis of non-dystrophic myotonias is usually achieved by targeted gene sequencing or whole exome sequencing. Using genome sequencing and a synergistic application of bioinformatic tools, we analyzed myotonic patients from 5 Saudi families in which standard genetic analysis did not reveal the genetic cause of myotonia.

Results: A deep intronic homozygous insertion located in intron 14 of the *CLCN1* gene was detected in the probands. Analysis of the inserted sequence revealed that it was a novel transmissible processed pseudogene, originating from the *UQCRH* gene. Its sequence presented all the typical features of these transposable elements: sequence similar to the parent gene, lack of all or most introns, presence of a poly-A tail and target site duplications at both ends. Patients with the pseudogene insertion showed a common haplotype suggesting a founder effect in the Saudi population.

Conclusion: The insertion of this non-reference processed pseudogene into intron 14 of *CLCN1* is likely a novel pathogenetic mechanism causing Becker recessive myotonia congenita that is not detected by conventional genetic testing.

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P12.039.C Whole genome sequencing and transcriptional profiling in patients with Duchenne muscular dystrophy carrying exon 2 duplication

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Background: Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by mutations in the *DMD* gene, 11% of which are duplications. Exon 2 duplication (Dup2) is the most common, typically associated with a severe phenotype.

Materials and Methods: Whole Genome Sequencing was performed on DNA from 21 DMD Dup2 patients, by Illumina DNA-PCR-Free-Prep-Kit and NovaSeq6000 platform. Full-length *DMD* isoforms transcriptional profile was evaluated in 12 DMD Dup2 muscle biopsies using 2100Bioanalyzer-High-Sensitivity-DNA-Kit.

Results: Genomic analysis showed that 20 of the Dup2 rearrangements were *in-tandem*, while 1 was anti-oriented. In 16 Dup2 cases, the 5' duplication breakpoint was in Purkinje intron 1, while in 5 cases it was in muscle intron 1. In 11 Dup2 rearrangements, breakpoints at the 5' or 3' ends were clustered, suggesting a potentially unstable genomic region. Transcriptional analysis revealed four splicing variants, of which the most represented was the exon 2 duplicated *out-of-frame* transcript. This splicing variant was driven by both brain (B) and muscle promoters in *in-tandem* Dup2. Notably and differently the main anti-oriented Dup2 transcript skipped both exons 2. Interestingly, 7 muscles revealed a moderate increase in B isoform expression, while one muscle displayed 51% of *wild-type* B transcript amount. The Purkinje isoform was undetectable in both DMD and control muscles.

Conclusion: All rearrangements are private and likely result from non-homologous-DNA-repair mechanisms. Transcription profile points out a possible role of *DMD* promoters in determining Dup2 splicing choices. This study enhances understanding of Dup2's impact on the *DMD* transcriptional pattern and may suggest new therapeutic strategies.

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P12.040.D Search for the causes of Duchenne/Becker muscular dystrophy in Russian patients without molecular diagnosis using whole genome sequencing

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Background: It is estimated that approximately 2–6% of cases Duchenne/Becker muscular dystrophy (DMD/BMD) are caused by complex structural rearrangements or deep intronic changes that are undetectable by routine diagnostic tests.

Material and Methods: WGS was performed on 16 unrelated Russian probands. The main inclusion criteria are an increase in the level of creatine phosphokinase (>6000 U/l), an established clinical diagnosis DMD/BMD, absence of molecular cause of the disease in previous conducted studies: MLPA and custom NGS panel

Results: In 81.2% (13/16) of patients, the cause of the disease was found. Variants in the *DMD* gene were detected in 53.8% (7/13): 2 probands had duplications missed earlier during diagnosis, 3 had pathogenic deep intron variants, 1 patient had a pericentric inversion of the chromosome X segment with a breakpoint in intron 7 of the *DMD* gene, and 1 patient had a balanced translocation between chromosomes 1 and X with a breakpoint in intron 48. In 46.2% (6/13), pathogenic variants in other genes responsible for muscular dystrophies were identified: *TK2, MICU1, SGCA, DYSF, ACTN2*.

Conclusion: This study showed an expansion of the diagnostic capabilities of muscular dystrophy using whole genome sequencing. Identification of pathogenic variants in the *DMD* gene is important in the aspect of application of etiotropic therapy.

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P12.041.A Etiology of a CHKB-deficient mouse model of muscular dystrophy: A platform for gene therapy development

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Background: Megaconial congenital muscular dystrophy (OMIM: 602541) is an autosomal recessive disorder caused by CHKB variants resulting in decreased choline-kinase-β activity, a key enzyme in phosphatidylcholine (PC) biosynthesis via the Kennedy pathway. Patients present with congenital or adolescent-onset limb-girdle muscular dystrophy, with some developing dilated cardiomyopathy and intellectual disability. Understanding disease pathogenesis and developing effective therapies require robust preclinical models.

Results: We investigated Chkb−/− mice as a disease model and therapeutic testing platform. Despite Chkb deficiency, PC levels

remained stable, while significant lipid metabolic disruptions occurred. Affected muscles exhibited impaired mitochondrial β-oxidation, leading to fatty-acyl-carnitine accumulation and subsequent fatty acid shunting into triacylglycerol and storage as lipid droplets. This metabolic shift correlated with reduced Ppar signaling. Ppar agonists restored fatty acid utilization, prevented triacylglycerol buildup, and upregulated Chka, a compensatory choline kinase isoform, in Chkb −/− myoblasts in vitro. Muscle integrity analysis showed an intact dystrophin-glycoprotein complex but disruption of the vinculin-talin-integrin system due to a-actinin detachment, impairing actin-sarcolemma connectivity. Choline kinase inhibition in U2OS cells induced PI(4,5)P2 internalization and reduced vinculin localization, reversible by Chkb overexpression. Chkb−/− mice exhibited cardiac hypertrophy, reduced left ventricular size, and arrhythmia susceptibility, mirroring patient phenotypes. Lipidomic analysis of heterozygous and homozygous Chkb−/− hearts identified elevated acylcarnitine, a known arrhythmogenic factor.

Conclusion: The Chkb−/− mouse recapitulates key aspects of CHKB-mediated muscular dystrophy and cardiac dysfunction, providing novel mechanistic insights into how defective PC synthesis drives disease pathology. These findings establish Chkb−/− mice as a valuable preclinical platform for developing therapeutic interventions for CHKB-mediated muscular dystrophy

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P12.042.B Genetic Findings in Over 500 Individuals Tested with a Spastic Paraplegia Gene Panel

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Background: Hereditary spastic paraplegia (HSP) is genetically heterogenous, with over 80 types identified to date. Despite the increasing knowledge, few studies have evaluated the yield of broad genetic testing in a large heterogenous cohort. We evaluated the yield of genetic testing in a cohort of individuals with suspected HSP.

Material and Methods: We performed a retrospective review of findings in a gene panel which included sequencing and copy number variant (CNV) analysis of up to 75 nuclear genes associated with spastic paraplegia.

Results: A total of 533 individuals underwent testing and 28.3% of them received an informative result. Adults were significantly more likely to receive a diagnostic result. Disease causing variants were reported in 25 different genes. Autosomal dominant inheritance was observed in 67.5% of the individuals, autosomal recessive inheritance in 29.1% and X-linked recessive inheritance in 3.3%. *SPAST* was the most frequent autosomal dominant gene and 17% of the variants in this gene were CNVs. *ABCD1* and *PLP1* accounted for all the X-linked cases. Variants in *CTNNB1*, *PAH*, and *C19orf12* were only seen in individuals tested at age 17 or younger. Overall, 11.3% of cases involved a CNV and non-coding variants contributed to <1% of informative results.

Conclusion: An informative result was received in approximately 28% of individuals. Adults were more likely to be given an informative result. Variants in *SPAST, SPG7*, and *SPG11* were the most common, in line with previous reports. The frequency of *SPAST* variants in autosomal dominant HSP cases was higher than previously reported.

C.O.I. Disclosure:

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P12.043.C Identification, characterization and segregation analysis of DM1 alleles containing non-CTG variant repeats (VRs) in a two-generation Italian family

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Background: Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic disease caused by a CTG repeat expansion in the 3′-UTR of *DMPK* gene. In the last years, an increasing proportion of DM1 alleles containing non-CTG variant repeats (VRs) have been identified, with still unclear molecular and clinical consequences. In this study, we analyzed a two-generation DM1 Italian family of DM1 patients carrying VRs in relation to phenotype, transmission and the methylation level of the DM1 locus.

Material and Methods: DM1 patients have been deeply analysed to assess DM1 mutation and phenotypes. VRs were characterized by a combination of TP-PCR, LR-PCR, Sanger and PacBio long-read sequencing (LRS).

Results: The two-generation family is composed by two brothers and their respective asymptomatic daughters carrying *DMPK* expanded alleles with VRs at 3′ end of the CTG expansion. The expanded alleles of the two daughters (248 CTG and 298 CTG) were fully sequenced and showed 4–5.3% of VRs consisting of CCGCTGCTG and CCG motifs. LRS sequencing is now ongoing to characterize the entire DM1 locus in both fathers (669 CTG and 729 CTG) also carrying CCGCTG and CCG motifs. A decrease in the methylation levels downstream of the CTG repeat tract has been observed across generations (from 26–38% to 8–17%), consistently with a contraction of paternally derived DM1 expansions.

Conclusion: Understanding the genetic and epigenetic variabilities of *DMPK* expanded alleles containing VRs in DM1 patients could implement clinical phenotype stratification and genotype-phenotype correlations, representing a useful tool in the DM1 genetic testing and counselling.

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P12.044.D Unraveling the complexity of mitochondrial DNA disorders: insights into Primary Mitochondrial Myopathies

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Background: A complex spectrum of phenotypes is associated with the plethora of mitochondrial DNA (mtDNA) pathogenic variants, going from isolated optic neuropathies to severe systemic encephalomyopathies. Among those, primary mitochondrial myopathies (PMM) are a relevant disease for both children and adults, affecting an average of approximately 5.5 in 100,000 people across Europe. The mtDNA variants leading to a PMM phenotype can be both SNVs and large somatic deletions/duplications. Moreover, a subset of the MM patients shows multiple deletions/duplications due to nuclear genes defects encoding enzymes of the mtDNA replisome.

Material and Methods: We analysed mtDNA from skeletal muscle biopsies derived from 100 patients with suspected mitochondrial dysfunction, through NGS sequencing at high coverage depth. We detected SNVs using the MutServe suite and large deletions/duplications with the MitoSAlt tool.

Results: We were able to identify several novel and already described pathogenic variants, located in both coding genes and tRNA sequences of mtDNA, as well as single and multiple macro-rearrangements. As expected, the most common genetic defects in our cohort are the m.3243A>G/*MT-TL1* and the single somatic deletions of mtDNA. This analysis also allowed us to assess the diagnostic yield of mtDNA sequencing across the cohort. Noteworthy, an increased yield is then achieved through analysis of WES or WGS in these patients.

Conclusion: Given their primarily mitochondrial etiology, we emphasize the outmost importance of in-depth analysis of mtDNA on the target tissue of PMM, especially using recent NGS approaches that can precisely define even the structural variants.

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P13 Multiple Malformation/Anomalies Syndromes

P13.001.A A distinct de novo frameshift variant in the last exon of *SATB1* causes Hyper-IgM syndrome with a neurodevelopmental phenotype (Award Candidate)

Yasmin Hamzavi-Abedi[1], ***Elke de Boer***[2], *Joery den Hoed*[3], *Maud Tusseau*[4], *Sandra von Hardenberg*[5], *Mathieu Fusaro*[6], *Caspar I. van der Made*[2], *Ulrich Baumann*[5], *Vincent Barlogis*[7], *Nicolas Chatron*[4], *Alexander Hoischen*[2], *Lisenka E.L.M. Vissers*[2], *Vincent R. Bonagura*[8]

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Background: Hyperimmunoglobulin M (HIGM) syndrome includes rare primary immunodeficiencies, characterized by defective class-switch recombination. This leads to normal or increased serum IgM, reduced or absent levels of IgG, IgA, IgE, and recurrent sinopulmonary infections. In approximately 25% of individuals the genetic cause remains unknown. SATB1 is a transcription factor that causes two overlapping neurodevelopmental disorders (NDD) through distinct pathophysiological mechanisms. In addition to neurodevelopment, SATB1 is crucial for immune function and lymphoid development.

Material and Methods: Through international collaborations, clinical and immunological data were collected from medical files of individuals with a distinct de novo frameshift variant in the last exon of *SATB1*, who presented with HIGM and syndromic NDD.

Results: Four unrelated individuals were identified, carrying the same de novo variant in *SATB1* (NM_002971.6:c.1877del;p.(Pro626-Hisfs*81)), who presented with NDD, seizures, dysmorphism and increased susceptibility to infections. All exhibited IgG and IgA deficiencies, with elevated IgM levels in three, and normal IgM in one. CD40L expression on activated CD4+ T cells as assessed by flow cytometry in three individuals was reduced. B cell phenotyping, performed in three individuals, showed deficiency in switched memory B cell numbers.

Conclusion: These cases implicate the c.1877del variant in *SATB1* in HIGM, which has not been described for any other *SATB1* variant so far. We propose *SATB1* as a cause for HIGM syndrome and suggest including *SATB1* in genetic diagnostic workup for primary immunodeficiencies. Further studies are needed to disentangle the pathophysiological complexity of the c.1877del variant, to elucidate its molecular consequences leading to immunological dysfunction.

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P13.002.B RENOVO-NF1 accurately predicts NF1 missense variant pathogenicity

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Background: Identification of a pathogenic variant in NF1 is diagnostic for neurofibromatosis, but is often impossible at the moment of variant detection due to many factors including allelic heterogeneity, sequence homology, and the lack of functional assays. Computational tools may aid in interpretation but are not established for NF1.

Material and Methods: We optimized our random forest-based predictor RENOVO for NF1 variant interpretation. RENOVO was trained and tested using an approach of "database archaeology": by comparing versions of ClinVar over the years, we defined "stable" variants that maintained the same pathogenic/likely pathogenic/benign/likely benign (P/LP/B/LB) classification over

time (n = 3579, the *training set*), and "unstable" variants that were initially classified as Variants of Unknown Significance (VUS) but were subsequently reclassified as P/LP/B/LB (n = 57, the *test set*). This approach allows to retrospectively measure algorithm accuracy on prediction with insufficient information, reproducing the scenario of maximal clinical utility. We further validated performance on: i) *validation set 1*: 100 NF1 variants classified as VUS at the time of RENOVO development and subsequently reclassified as P/LP/B/LB in ClinVar; ii) *validation set 2*: 15 de novo variants discovered in a prospective clinical cohort and subsequently reclassified per ACMG criteria.

Results: Accuracy was 98.6% on the training test, 96.5% in the test set, 82% in validation set 1 (but 96.2% for missense variants) and 93.7% on validation set 2.

Conclusion: RENOVO-NF1 accurately interprets NF1 variants for which information at the time of detection is insufficient for ACMG classification, and may overcome diagnostic challenges in neurofibromatosis.

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P13.003.C Classification of missense DNMT3A variants using a newly derived blood DNA methylation signature for Tatton-Brown Rahman syndrome

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Background: Pathogenic variants in *DNMT3A* are causative of Tatton-Brown-Rahman syndrome (TBRS), a disorder characterized by overgrowth, distinctive facial dysmorphia, and intellectual disability. Our previous work has shown that syndromic conditions caused by pathogenic variants in epigenetic regulatory genes have identifiable patterns of DNA methylation (DNAm) change termed: DNAm signatures. Given the role of *DNMT3A* as a de novo DNA methyltransferase, we investigated whether variants underlying TBRS are associated with a DNAm signature.

Methods: We profiled whole-blood DNAm for 45 individuals with DNMT3A variants and 71 typically developing individuals using Illumina's Infinium MethylationEPIC v2.0 microarray.

Results: We identified a robust DNAm signature of 443 CpG sites (FDR < 0.05; $\Delta\beta > 20\%$) in 7 individuals with TBRS and validated it in an additional 20 individuals with TBRS. To demonstrate the diagnostic utility of the signature we classified the DNAm profiles of 9 individuals with variants of uncertain significance in DNMT3A. We found that TBRS-causing variants in DNMT3A result in different magnitudes of methylation change, with 7 individuals exhibiting mild hypomethylation and 3 individuals exhibiting severe hypomethylation. We also classified the DNA methylation profiles of 7 individuals with Heyn-Sproul-Jackson syndrome also caused by pathogenic variants in DNMT3A.

Conclusion: In this study we generated a TBRS specific DNAm signature and demonstrated its diagnostic utility in classifying uncertain variants in *DNMT3A*. Our findings also highlight the complex genotype-phenotype-epigenotype relationships underlying TBRS. Future work is focused on elucidating these relationships and expanding our understanding of the epigenetic landscape underlying disease causing variants in *DNMT3A*.

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P13.004.D First patient cohort with a novel syndromic neurodevelopmental disorder caused by the c.231+4A>C hotspot variant in the *LSM1* gene

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Background: The *LSM1* gene encodes a subunit of the conserved LSM1-7 protein complex involved in mRNA metabolism. Variants in *LSM1* gene have been described in two separate case reports. The first published report identified the homozygous splice-site variant c.231+4A>C, while the second reported a homozygous missense variant. Nevertheless, variation in *LSM1* has yet to be established as disease-causing in humans.

Material and Methods: Through exome sequencing and detailed phenotyping, we report six syndromic pediatric patients with the homozygous c.231+4A>C variant in the *LSM1* gene, collected via GeneMatcher. GestaltMatcher was used to analyze facial feature similarity and real time quantitative PCR (RT-qPCR) confirmed the splice defect caused by the variant. Haplotype analysis assessed whether this variant resulted from independent occurrences or a common ancestral haplotype.

Results: Patients presented with dysmorphic facial features, developmental delay, and multi-systemic involvement, including urological, cardiac, and skeletal manifestations, showcasing the phenotypic spectrum of this syndrome. RT-qPCR confirmed that the c.231+4A>C variant causes exon 3 skipping, producing negligible *wild-type LSM1* mRNA expression. Elevated mutant isoform expression confirmed pathogenicity according to the ACMG guidelines. We identified this variant in the Muslim Arab and Ashkenazi Jewish populations and determined that it represents a hotspot variant through haplotype analysis.

Conclusion: Our findings establish *LSM1*, and specifically the c.231+4A>C homozygous variant, as causative for a novel autosomal recessive syndromic neurodevelopmental disorder. These results expand the understanding of *LSM1*-related diseases and provide a foundation for further investigation of its molecular mechanisms.

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P13.005.A Loss-of-function variants of *ZFTRAF1*, encoding a novel autophagy regulator, cause neurodevelopmental disorder of microcephaly, hypotonia, and global developmental delay

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Background: Neurodevelopmental disorders are a group of conditions characterized by manifestations of brain dysfunction, leading to impairments in social, behavioral, and cognitive functioning.

Material and Methods: Here, we studied five patients from three unrelated families originating from Yemen, Germany, and Spain. Variants were identified by exome-sequencing and homozygosity mapping and their consequences were investigated using immunofluorescence, immunoblotting, pulldown assay, and RNA-sequencing.

Results: The affected individuals manifested global developmental delay, variable degrees of microcephaly, and hypotonia. Genomic analyses revealed the biallelic variants in *ZFTRAF1*, encoding a protein of unknown function to date. We detected two frameshift variants in two families and an intronic splice site variant in the third family. We investigated ZFTRAF1, the poorly characterized protein, at the cellular level and signified it as a nucleocytoplasmic protein in different human cell lines. We also investigated the ZFTRAF1 interactome by pulldown assays and identified 110 interacting proteins that are crucial components of the mRNA-processing machinery and autophagic mechanism. ZFTRAF1 was completely absent in primary fibroblasts derived from two independent patients. Profiling of autophagy markers, as well as whole-transcriptome and whole-proteome analyses, revealed dysfunctional autophagy in patient-derived fibroblasts. Another evidence of impaired autophagy was the fact that attempts to reprogram patient fibroblasts got stuck in the pre-iPSCs phase.

Conclusion: Thus, our findings suggest that biallelic variants of *ZFTRAF1* cause a severe neurodevelopmental deficiency syndrome. En masse, the loss of its function leads to inoperative autophagy and mRNA processing, both of which are known to have crucial roles in normal human brain development.

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P13.006.B Single-cell multi-omics RNA-seq analysis provides new insights into pathomechanisms of Mulvihill-Smith-syndrome

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Background: Mulvihill-Smith-syndrome (MSS) is a nano-rare genetically unsolved progeroid disorder with only few patients described so far. We report preliminary data on immunological and gene expression profiles of a 16-year-old MSS patient.

Material and Methods: multi-omics RNA-seq (gene expression, surface protein immune cell markers, TCR, BCR) analysis was applied on single-cell suspensions of PBMCs of the patient and her mother (control). After single cell RNA preparation according to Chromium Next GEM Single Cell 5'-Reagent Kits_v2 (10XGenomics) protocol, gene expression matrices were assessed with the Seurat 5.01 package in RStudio.

Results: The patient showed, compared to control, increased CD4+/CD8+ T cells, expansion of paired αβ TCRs and reduction in naive T cells, indicative of a progressive aging phenotype. Neither diabetes, recurrent infections or cancer were present at the time of the investigation. Pathway analysis revealed a higher inflammatory response of $CD4^+$ EM T cells (overexpression of *SOCS2, PTGDS, TNFRSF4, PTGER2)* and increased cell-killing potency of $CD8^+$ EM T cells *(*upregulation of *CD28, GZMK*, and *PDCD1)* in the MSS patient. Moreover, several pathways related to integrin-mediated signaling, response to hormones, fatty acid metabolism, response to toxic substances and transcriptional processes were differentially regulated in $CD4^+$ EM T cells.

Conclusion: Single cell multi-omics RNA-seq analysis confirmed an aged immune system and dysregulation of several pathways in our patient. This technique seems to be a valuable approach to simultaneously study rare diseases at cellular and molecular level. We are interested in further characterizing MSS patients. For potential collaborations please contact olaf.riess@med.uni-tuebingen.de

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P13.007.C A reverse phenotyping approach to identify pathogenic *SMARCA4* variants causing Coffin-Siris syndrome through integration of clinical, genomic, and structural data

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Background: Coffin-Siris syndrome (CSS) is a developmental disorder characterized by distinctive facial features, hypoplasia of the fifth finger, developmental delay, and intellectual disability. CSS is caused by pathogenic variants in subunits of the BAF chromatin-remodeling complex, which are essential for neurodifferentiation. Despite advancements in genetic diagnostics, a significant number of CSS cases remain unresolved, highlighting the need for further research.

Material and Methods: We utilized a reverse phenotyping approach to identify CSS-related variants in BAF complex genes. We first analyzed clinical and whole-exome sequencing data from 6,189 patients. We focused our study on the *SMARCA4* gene. Variants were filtered based on allelic frequency, pathogenicity scores, and read depth. Finally, mechanistic data for how each potentially pathogenicity variant alters the structure, stability, or flexibility of the BAF complex, was evaluated using computational structural genomics. This approach leveraged information from multiple experimental structures to generate a structure-function mapping. In-depth phenotyping is currently being conducted by clinical geneticists and pediatric neurologists.

Results: We identified 56 unique SMARCA4 missense variants in 53 patients. Among patients with a complete clinical record, three were undiagnosed and exhibited facial dysmorphisms along with intellectual disability. Preliminary protein complex-based structural calculations indicated that these variants might disrupt the assembly of the BAF complex, leading to a reevaluation of the clinical phenotype.

Conclusion: Reverse phenotyping may enhance the diagnosis of neurodevelopmental disorders such as CSS. By integrating structural bioinformatics, systems biology, and computational biology, we can improve the interpretation of genomic data in the diagnosis of rare diseases.

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P13.008.D Dissecting the clinical variability observed in HNF1β associated phenotypes

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Background: HNF-1β is a transcription factor involved in early development of organs as kidney, liver and pancreas. HNF1β mutations have been associated with a broad spectrum of abnormalities including Maturity Onset Diabetes of the Young type 5 (MODY5), Renal Cysts And Diabetes (RCAD), congenital anomalies of kidneys and genitourinary tract, liver only involvement, neoplastic condition.

Material and Methods: We selected three mutations: a missense mutation (c.436A>G, p.Asn146Asp) in a familial case of RCAD; a frameshift mutation (c.712delT, p.Trp238GlyFs*25) in a familial case with only renal cysts and a missense mutation (c.1484T>A; p.Met495Lys) in a familial MODY5 case.First, we generated a HNF1β KO in HK2 cells and through lentiviral production we established HA-labelled stable cells expressing selected HNF1β mutations or the wild type gene.

Results: Mutations affect expression and size of the HNF1β protein. Furthermore, HNF1β regulates genes involved in renal cystogenesis and in biogenesis and function of mitochondria. HNF1β mutations lead to increase in ROS production, morphological alterations of mitochondria and change in their membrane potential with a metabolic switch towards glycolysis. Transcriptomic analysis revealed alterations in gluconeogenesis, glycogen synthesis, response to insulin stimuli and branching of epithelial morphogenesis.

Conclusion: Our results indicate that HNF1β mutations cause downregulation of different transcripts according to the phenotype studied. The mitochondrial phenotype was observed in cells expressing the renal cysts only and RCAD phenotypes. We believe that our study will identify the molecular mechanisms underlying the phenotypic variability observed in the HNF1β associated clinical spectrum.

C.O.I. Disclosure:

F. Massaro: None.

P13.009.A Genotypes heterogeneity in PIK3CA-related overgrowth spectrum (PROS)

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Background: Postzygotic somatic variants in cancer-associated genes drive various overgrowth and vascular malformations syndromes, including PIK3CA-related overgrowth spectrum (PROS). PROS comprises over 20 conditions characterized by asymmetric overgrowth and vascular anomalies. Mutations arise during embryogenesis, leading to mosaic mutation distribution. The clinical variability of PROS is influenced by affected tissue type, mutation timing, PI3K activation and additional factors. The overlap with other syndromes and the complexity of detecting mosaic variants pose significant diagnostic challenges.

Material and Methods: 120 patients from unrelated families clinically diagnosed with PROS underwent molecular genetic testing by deep NGS (>1000x) of a targeted panel of genes associated with overgrowth syndromes and vascular malformations. DNA was extracted from fresh-frozen postsurgical material, skin biopsy or FFPE of the pathological lesion.

Results: Pathogenic or likely pathogenic variants were identified in 75 patients (62,5%). A single variant was detected in 72 patients, primarily in *PIK3CA* (n = 45), followed by *GNAQ* (n = 7), *RASA1* (n = 5), *PTEN* (n = 3), *AKT1* (n = 3), *TEK* (n = 2), *PIK3R1* (n = 2), and one case each in *KRAS*, *NRAS*, *HRAS*, *BRAF*, *AKT3*, *GNA11*, and *MAP3K3*. The coexistence of two clinically significant variants was observed in 3 cases.

Conclusion: Molecular genetic testing is essential for the diagnosis and treatment of PROS. The identification of PIK3CA variants provides the basis for prescribing PI3K-inhibitor alpelisib.

Our findings emphasize the genetic heterogeneity of PROS and related disorders, highlighting the necessity of comprehensive genetic testing for accurate diagnosis and potential targeted therapy selection. In cases where PIK3CA variants are not detected, alternative therapeutic strategies must be considered.

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P13.010.B CDK13-related disorder: clinical spectrum and clinical management recommendations from a cohort of 27 novel patients

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Background: In 2016, Sifrim et al. described the first patients carrying a variant in *CDK13* and with a common clinical picture characterized by congenital heart defects, intellectual disability and peculiar facial dysmorphisms (Congenital Heart Defects, Dysmorphic Facial Features, and Intellectual Developmental Disorder; CHDFIDD. OMIM # 617360) generally referred to as *CDK13*-related disorder. The specific phenotypic spectrum is still under definition.

Material and Methods: We describe 27 patients, hitherto unreported, collected through a multicenter effort, all carrying a pathogenic or likely pathogenic variant in *CDK13*.

Results: Besides the most common features, we found some novel findings: cranyosinostosis and variable skeletal features (e.g. cranio-cervical anomalies). We also describe the ocular phenotype that includes bilateral congenital glaucoma, posterior embriotoxon, buphthalmos and Duane anomaly. Moreover, we observed a case of mother-to-daughter transmission, an exceptional event due to the fact that for affected patients the chance of having a child is generally considered to be low. We also saw a pair of monozygotic twins that shared the same variant and an almost identical clinical picture. Furthermore, we highlight the possible endocrinological issues that comprise obesity with insulin-resistance and delayed puberty.

Conclusion: Our report adds some novel features of *CDK13*-related disorder, whose phenotypical spectrum varies from mild neurodevelopmental disorder and behavioral issues without other recurrent malformations to a more severe clinical picture. We also reviewed the literature and summarizing these data with our clinical findings, we redefined the differential diagnosis and we added some recommendations for the clinical approach and the follow-up care of *CDK13*-related disorder.

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P13.011.C Pain of muscle-skeletal origin, muscular fibro-adipose involution and impaired muscle fiber differentiation as crucial features in Costello syndrome

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Background: Costello syndrome (CS) is a rare condition belonging to RASopathies characterized by musculoskeletal anomalies and pain, decreased muscle force. Anecdotal papers reports muscle fibers anomalies in in vitro cellular models. Herein, we studied skeletal muscle architecture and fibro-adipose infiltration (FAI) in CS by Ultrasonography (US) and assessed the *HRAS* involvement in skeletal muscle defects using engineered myoblasts cell lines expressing two recurrent *HRAS* variants(p.Gly12Ser and p.Gly13Cys).

Methods: 20 CS individuals were enrolled. Paravertebral and lower limb muscles were studied by US (Samsung RS85 Prestige). FAI was graded according to the Heckmatt scale (I-IV). Myoblasts cell lines expressing CS variants were generated by retroviruses infection and purification by flow-cytometric sorting.

Results: FAI was identified in at least one district in all participants. A grade II-III FAI was detected in lumbar paravertebral muscles (85%) and lower limbs (at least one examined district in 75% indivuduals), with vastus lateralis being the most frequently involved. Myoblast cell lines expressing HRAS variants showed impaired muscle differentiation capabilities due to compromised myoblasts cell cycle, decreased expression of late muscle specific markers, and defective fusion into multinucleated myotubes. Moreover, immunofluorescence analyses performed on these cell lines identified lipid droplets content confirming data obtained on patient's fibroblasts by Carpentieri et.al.

Conclusions: Muscle FAI represents a new feature of CS probably correlated to pain and reduced muscle strength. Considering the high fat content in patient's cells and myoblasts model, we speculate that *HRAS* dysregulation causes a metabolic dysfunction leading to increased adipose generation and storage in CS-skeletal muscles.

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P13.012.D Large-scale Simons Searchlight medical & genetic data available across 180 rare genetic neurodevelopmental disorders (NDDs)

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Background: The international Simons Searchlight research program (**SimonsSearchlight.org**) has collected cross-sectional and longitudinal caregiver/participant-provided medical, developmental, and behavioral data and biospecimens for 180 genetic neurodevelopmental disorders. Participants can be re-contacted for additional research participation through SFARI Research Match. Researchers can request data and biospecimens for analysis and publication via SFARI Base (www.base.sfari.org).

Material and Methods: Simons Searchlight has enrolled >3000 affected participants with 156 monogenic and 24 copy number variant conditions from 71 countries. Medical history (n = 2,502), seizures (n = 2,361), development and behavior (n = 2,461), sleep (n = 1,576), quality of life (n = 1,580) data, and more, are available. 120 patient-derived induced pluripotent stem cell lines are available, with 80 more in progress. Here, we investigated the comorbidity of neurodevelopmental issues across genetic conditions.

Results: Linear regression testing of frequencies of select medical issues, calculated per genetic condition group having >5 likely/pathogenic carriers, was conducted using 2,242 medical histories. The results suggest moderate correlations between frequencies of intellectual disability (ID) and a language disorder, autism spectrum disorder (ASD) and ADHD, and movement disorders (eg, ataxia, tremors) and inability to walk. Results suggest a weak relationship between ID and seizures, hypotonia and movement issues, and hypotonia and gastrointestinal issues. Data suggest moderate and weak inverse associations between hypotonia and ASD, and ID and ASD, respectively.

Conclusion: Our analysis suggests relationships between certain diagnoses across a diverse neurogenetic cohort. Researchers can request data and biospecimens, and submit Research Match applications, at www.base.sfari.org.

Linear regression for the frequency of select medical issues across genetic conditions.

Comparison	R2	F(1,72)	p
ID and language	.32	33.72	< .001
ASD and ADHD	.23	22	< .001
Movement issues and inability to walk	.35	37.94	< .001
ID and seizures	.084	6.64	= .012
Hypotonia and movement	.12	9.88	= .002
Hypotonia and gastrointestinal	.068	5.26	= .025
Hypotonia and ASD	.18	16.28	< .001
ID and ASD	.000024	0.0017	= .967

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P13.013.A Acute kidney injury and chronic kidney disease in individuals with Down syndrome: A nationwide cohort study

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Objectives: In this nationwide cohort study, we investigated the risk of acute kidney injury (AKI) and chronic kidney disease (CKD) in individuals with Down syndrome (DS) compared to the general population.

Material and Methods: We identified a population-based cohort of individuals with cytogenetically confirmed DS from 1961–2021 using the Danish Cytogenetic Central Registry. For each individual with DS we sampled 10 age-and-sex-matched individuals without DS from the general Danish population. We used laboratory data on creatinine to assess AKI and CKD overall and by different stages and computed the cumulative incidence (risk) of AKI and CKD by age. Furthermore, we estimated the risk of AKI and CKD in subjects without congenital heart disease (CHD).

Results: We identified 2,815 individuals with DS with available laboratory data on plasma creatinine measurements. Comparing individuals with DS to the matched cohort, the risk of AKI was 28.9% vs. 1.4% at age 20, 32.8% vs. 5.3% at age 40, and 48.6% vs. 23.8% at age 70. The risk of CKD was 1.2% vs. 0.1% at age 20, 3.9% vs. 0.4% at age 40, and 23.2% vs. 13.8% at age 70. For subjects without CHD the risk AKI and CKD decreased but remained considerably higher for individuals with DS than for matched individuals.

Conclusion: We found that individuals with DS were at increased risk of developing both AKI and CKD compared to the general population. Additionally, co-occurring CHD could not fully explain the increased risk of AKI and CKD in individuals with DS.

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P13.014.B Variant spectrum and genotype-phenotype correlations in *HNRNPK*-related disorder (Au-Kline-Okamoto syndrome)

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HNRNPK-related disorder, also known as Au-Kline syndrome or Okamoto syndrome (AKS, OMIM 616580) is a multiple malformation syndrome associated with intellectual disability and recognizable dysmorphic features. The first individuals described had de novo loss of function variants in *HNRNPK*. Pathogenic missense variants are now also reported, with the previously identified genome-wide methylation signature supporting variant classification. *HNRNPK* encodes for heterogeneous ribonucleoprotein K (hnK), which is implicated in RNA metabolism. HnK has two N-terminal KH domains and a C-terminal KH domain, which are implicated in RNA binding. We report on the spectrum of clinical features of over 60 individuals with likely pathogenic or pathogenic variants in *HNRNPK*. We include more comprehensive characterization of prenatal phenotypes as provide an update on the frequency of malformations and emerging health issues. We identify several recurrent missense variants (eg. p.Glu85Lys; variants at p.Arg46) and observe pathogenic missense variants cluster in N-terminal KH domains and predicted truncating variants cluster in the C-terminal region. Missense variants correlate with lower malformation burden when compared to predicted truncating variants. We also report a significant association with earlier attainment of motor milestones for individuals with missense variants. We describe a potentially distinct phenotype for 2 individuals with variants at the G-X-X-G nucleic acid binding motif in the KH1 domain. Future work includes an expanded natural history study with longitudinal data collection to better understand the impact of genotype on outcomes as well as cell-based functional work to understand the impact of variants on HnK functional domains and RNA binding.

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P13.015.C Burden of common clinical conditions in adults with 22q11.2 microdeletion

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Background: Limited information is available on adults with rare genetic diseases. We investigated the clinical burden of commonly associated conditions in adults with a 22q11.2 microdeletion.

Material and Methods: In a cohort of 405 adults with 22q11.2 microdeletion (52% female, median age 30.6, IQR 24.8–41.2, years) we evaluated lifetime health records to identify conditions present in ≥5% that required assessment by a specialist and/or significant/ongoing management in adulthood. We used regression modelling to identify predictors of the number of conditions per individual, accounting for age, sex, ancestry, and conditions likely to affect burden: major congenital heart disease (CHD), schizophrenia, and moderate/severe intellectual disability (ID).

Results: We identified 66 conditions from 12 organ systems to be common in 22q11.2 microdeletion, with a median of 12 (range 1–31) conditions involving 7 systems per individual. Those with shorter proximal nested deletions had fewer conditions overall than those with typical 2.5–3.0 Mb 22q11.2 microdeletions ($p = 0.02$). Transmitting parents had more conditions. Older age ($p < 0.0001$), schizophrenia ($p < 0.0001$), major CHD ($p = 0.0005$) and moderate/severe ID ($p = 0.02$), but not sex or ancestry, were significant independent predictors of clinical burden (model R2 0.30, $p < 0.0001$). Most individuals ($n = 346$, 85%; median age 32.1) had one or more of 11 conditions associated with aging.

Conclusion: By early adulthood, the global burden of multisystem conditions associated with 22q11.2 microdeletion is apparent, emphasizing the need for multidisciplinary care for this population, and confirming full penetrance. The results suggest that the 22q11.2 microdeletion can be seen as a model for premature aging.

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P13.016.D Size matters: phenotypes associated with nested chromosome 22q11.2 deletions

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Background: 22q11.2 deletion syndrome (22q11.2DS) results from non-allelic homologous recombination due to low copy repeats (LCRs) A, B, C, and D. Most deletions extend from LCR22A-LCR22D (A-D) and include FISH probes within A-B and important developmental genes *TBX1* (within A-B) and *CRKL* (within C-D). Here we report deletion size and associated features in a large cohort towards establishing genotype-phenotype correlations.

Methods: Findings in 1667 patients with 22q11.2DS were examined under IRB approval. 328 diagnosed by FISH were excluded.

Results: 82% had A-D deletions (1095/1339). 4.7% were not mediated by LCRs. 6.6% would be missed by FISH. Prevalence of congenital heart disease in those deletions including *TBX1* was statistically significantly higher compared to those not including *TBX1* ($p < .01$): **A-D** deletions ($n = 1095$; 66%); **A-B** ($n = 69$; 73%); **A-C** ($n = 24$; 76%); **B-D** ($n = 57$; 26%); **C-D** ($n = 31$; 38%). Hypocalcemia was present in: A-D (52%), A-B (27%), A-C (42%), B-D (2%), C-D (0%). Chronic infection was equal across deletions. Patients with C-D deletions had no palatal nor structural brain anomalies, likewise scoliosis (8%), idiopathic seizures (3%), and ADHD (3%) were uncommon. Mean FSIQ by deletion type revealed: A-D (77.3; $n = 312$), A-B (87.8; $n = 16$); A-C (81.0; $n = 6$); B-D (78.3; $n = 5$); and C-D (87.7; $n = 3$) but numbers were small across nested deletions and standard deviations wide-ranging. 14% (397/461) of A-D deletions were familial, compared with A-B (22%), A-C (17%), B-D (71%), and C-D (67%).

Conclusions: Deletion size significantly impacts phenotypes and is key in providing personalized healthcare guidance, identifying causative genes, and providing recurrence risk counseling.

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P13.017.A Genome sequencing of unsolved patients confirms genetic heterogeneity in Cornelia de Lange syndrome

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Background: Cornelia de Lange syndrome (CdLS) is a rare neurodevelopmental disorder primarily caused by mutations in various subunits or regulators of the cohesin complex. However, 30% of patients remain without a molecular diagnosis. In line with the role of cohesin in genomic integrity and DNA accessibility, CdLS falls within the category of transcriptomopathies, a group of disorders characterized by global transcriptional dysregulation.

Material and Methods: Trio whole genome sequencing was performed to investigate 15 unsolved individuals with CdLS-like phenotypes. For all patients, germline or mosaic variants in known CdLS-associated genes had been previously excluded.

Results: Putative disease-causing variants in non-CdLS genes were identified in six patients: two heterozygous missense variants (*DDX23, CSNK1G1*), one hemizygous nonsense variant (*CDK16*), two heterozygous in-frame deletions (*MAU2*, *CREBBP*), and one homozygous missense substitution in *TMTC3*. Additionally, one intragenic deletion affecting *USP7* and one intragenic duplication of *AFF2* were detected. A subsequent episignature study conducted on the remaining unresolved cases identified a CdLS-specific methylation profile in one patient. Re-analysis of the genome data of this patient revealed a de novo deep intronic single nucleotide exchange in *NIPBL*. Blood RNA analyses confirmed aberrant splicing of the *NIPBL* transcript. The genome data of the six remaining unsolved cases are still under investigation. Systematic screening for alterations in repeat expansions identified a possible expansion within the *AFF3* gene promoter for one patient.

Conclusion: The newly identified variants affect proteins involved in transcriptional regulation, confirming the genetic heterogeneity of CdLS and supporting its classification as transcriptomopathy.

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P13.018.B Dissecting Pitt-Hopkins syndrome into independent molecular categories, and first evidence of genetic heterogeneity of the condition: analysis of a large cohort of patients

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Background: Pitt-Hopkins syndrome (PTHS) is a neurodevelopmental disorder with a distinctive clinical phenotype including severe intellectual disability (ID), absent speech, breathing anomalies and typical facial features. The only known causative gene is *TCF4*. Recently, both a wider spectrum of *TCF4*-associated phenotypes and a possible genetic heterogeneity for PTHS have emerged.

Material and Methods: We present a cohort of 53 patients with a clinical diagnosis of PTHS and a detected genomic defect, affecting mainly *TCF4*, but also other genes. Including literature revision, we aimed to define an intragenic syndromology of *TCF4*,

and to explore the hypothesis that other genes may be responsible for classical PTHS. Genetic analyses included array-CGH, MLPA and NGS; standard karyotyping, optical genome mapping and cDNA studies in selected cases.

Results: We observed a full PTHS phenotype, as per consensus clinical score, in 47 patients: 45 with variants disrupting the coding sequence between exons 9 and 19 of *TCF4*, including a 2 Mb inversion; 2 with pathogenic variants in *SOX11* and *HECW2*. The remaining 6 patients presented with milder ID and good language ability (atypical/intermediate PTHS?), caused by truncating variants in exons 7–8, by some missense variants, or by variants limited to exons 1–6 of *TCF4* but associated with deletions of the upstream regulatory region.

Conclusion: Molecular pathology of the *TCF4* gene leading to different phenotypes in the spectrum of PTHS and definite evidence of genetic heterogeneity of the classical PTHS are discussed here for the first time.

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P13.019.C Abnormal growth in Epigenetic Machinery diseases: Energy Expenditure reveals an unexplored metabolic link

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Background: Mendelian disorders of the epigenetic machinery (MDEMs), include a large number of conditions. Their main clinical findings are multiple congenital anomalies and intellectual disability together with growth dysregulation, that can be variably up- or down-regulated. In addition, in several MDEMs a predisposition to metabolic syndrome and childhood obesity has been reported.

Material and Methods: A heterogeneous pool of 35 patients affected by MDEMs was engaged in this study. The most frequently represented syndromes are Rubinstein-Taybi and Sotos ones. We divided our cohort into 2 subgroups: those exhibiting growth failure (A) and those displaying an overgrowth (B). For each patient we collected physical data and performed indirect calorimetry as a measure of Resting Energy Expenditure (REE). A comparison of REE was made between the two subgroups and versus age-, sex-, height-matched healthy controls.

Results: Notably, the Group B demonstrated a significantly higher REE compared to both Group A both controls. This difference was height-independent. On the other hand, REE measured in Group A presented no significant differences compared to the controls.

Conclusion: A first analysis of energy expenditure in MDEMs was performed, its results demonstrate the presence of an impaired metabolism in patients diagnosed with chromatinopathy characterized by overgrowth. They also suggest an etiopathogenetic mechanism that may go beyond overgrowth itself, paving the way to future studies that will deepen the knowledge about energy metabolism andinvestigate the metabolic bases of this abnormal growth.

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P13.020.D Optimizing Cornelia de Lange syndrome diagnosis through clinical scoring and next-generation phenotyping integration

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Background: Cornelia de Lange Syndrome (CdLS) is a rare multisystemic developmental disorder with variable phenotype and overlapping features with multiple syndromes. This study combines the CdLS clinical score with a facial phenotype-based score generated through next-generation phenotyping (NGP) to evaluate the phenotypic spectrum of CdLS patients.

Methods: Molecular testing was performed for 85 patients with CdLS diagnosis hypothesis: 48 were diagnosed with CdLS, 18 had other syndromes, three had variants of uncertain significance (VUS), and 16 remained undiagnosed. The clinical score classified patients' phenotype as classic, non-classic, or only indicative of molecular testing for CdLS. The GestaltScore used GestaltMatcher's evaluation of patients' photos to quantify the likelihood that a genetic variant was pathogenic, assigning evidence to the ACMG PP4 criterion.

Results: All patients diagnosed with CdLS had at least a moderate evidence level of PP4 assigned by the GestaltScore. Among these, those with classic or non-classic phenotypes had at least moderate PP4. Interestingly, the ones with only molecular indication for CdLS testing had strong or very strong PP4. One VUS had very strong evidence for PP4 and was reclassified as likely pathogenic. Among the patients diagnosed with other syndromes, the ones with only indicative molecular testing for CdLS had a moderate or lower PP4 evidence level. Undiagnosed patients' phenotypes ranged across the three clinical categories and had at least moderate PP4.

Conclusion: Combining clinical scoring with NGP enhances clinical accuracy for CdLS, particularly in milder phenotypes, and facilitates reclassification of VUS, supporting further decisions on molecular testing in challenging cases.

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P13.021.A Clinical delineation and genotype-phenotype correlation in 104 pediatric patients with Kabuki syndrome: a Large longitudinalCohort from a Single Center in China

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Background: Kabuki syndrome is a monogenic genetic disorder affecting multiple systems, with an incidence rate of 1 in 32,000 to 1 in 86,000. This study provides a detailed genotype and phenotype analysis of a large longitudinal cohort of Kabuki Syndrome (KS) from a single center in China.

Material and Methods: From July 2017 to July 2024, participants were enrolled at Shanghai Children's Medical Center. Variants in KMT2D or KDM6A were identified through whole exome sequencing. Phenotype data included prenatal and perinatal history, neonatal, childhood and adolescence evaluations.

Results: A total of 104 KS individuals fullfiled 362 outpatient visits, with an average follow-up of 2.58 years. Growth curves were plotted based on 545 follow-up height data points. Among the patients, 27.08% had congenital heart defects (CHD), and 3 patients were identified with anomalous pulmonary venous connection as a new KS phenotype. KS patients showed facial feature heterogeneity, patients with atypical facial features associated with a older diagnosis age and a more diverse and severe phenotype. The Face2gene software correctly identified the facial photographs of all 85 KS patients.

Conclusion: This study offered a comprehensive description of a Chinese KS cohort. And provided the first growth curves for Chinese KS patients and a detailed CHD phenotype spectrum. Our findings also suggest that artificial intelligence (AI) -assisted facial diagnostic criteria will be a valuable in the clinical diagnosis of KS.

C.O.I. Disclosure:

W. Yirou: None.

P13.022.B Clinical and Molecular Characterization of Pierre Robin Sequence in WGS era

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Background: Pierre Robin Sequence (PRS) defines the association of micrognathia, glossoptosis, and respiratory difficulties. Recent European guidelines (ERN CRANIO 2024) suggest that even in apparently isolated PRS, genetic testing should be included in the diagnostic process, although the etiological cause remains unknown in most cases.

Material and Methods: 50 subjects diagnosed with PRS were enrolled and studied clinically and molecularly. For subjects without an etiological identified cause, WGS or reanalysis of WES data was performed. Virtual gene panels for novel candidate genes, based on animal models or function, were applied and non-coding regions on either side of *SOX9* were investigated.

Results: At birth, 25 subjects were classified as isolated PRS, but 13 were later reclassified as non-isolated during follow-up. 25 subjects presented a complex phenotype from birth. A genetic cause was identified in 20 out of 50 subjects (40%). No pathogenic variants in novel candidate genes were found. ArrayCGH showed a detection rate (DR) of 6%, while WES and WGS had higher DRs (34% and 40%, respectively). Among the 25 cases initially classified as isolated PRS, WES and WGS would have had a DR of 16%, identifying early two cases of Stickler Syndrome and two of Campomelic Dysplasia.

Conclusion: Genetic characterization of PRS, even in isolated cases, is essential for identifying rare genetic causes, refining prognosis, optimizing treatment, and improving family counseling. WES/WGS as a first-line diagnostic tool is recommended. The DR of WGS is expected to increase, enhancing the interpretation of intronic variants and further improving diagnostic accuracy.

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P13.023.C Diagnostic analysis through genome sequencing of a cohort of 69 Patients with syndromic or recurrent neural tube defect

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The France Genomic Medicine Plan 2025 allows for the sequencing of genomes from patients with rare diseases in France, through 77 pre-indications, including dysraphisms, particularly syndromic dysraphisms or those with intra-familial recurrence.

A total of 69 cases were analyzed using genome sequencing. In this cohort, there is a female predominance (40/69, 58%), as reported in the literature. 59.4% (41/69) were fetuses, while 40.1% (28/69) were children or adults. In 24.5% (17/69) of cases, a family history was observed. Most of the neural tube defects were lumbar (53/69, 79.7%), 14.5% (12/69) involved the cephalic pole, and 5.8% (4/69) were craniorachischisis or inencephalies. In 78.9% (54/69) of cases, associated malformations were present. The most common were vertebral anomalies (24.6%), renal anomalies (17.4%), and limb anomalies (17.4%).

Regarding molecular results, a 22q11.2 microdeletion and a truncating inherited variant in *TBXT* were identified, both of which correspond to risk factors for dysraphism. A likely pathogenic missense variant was found in *WBP11* in a patient with a VACTERL-like syndrome. A candidate variant was also identified in *PRICKLE1*, which is involved in planar cell polarity. Additionally, variants were found in candidate genes such as *ZIC1*, *ZMYM3*, and *KIAA0556*.

The exploration of these genomes was conducted within a diagnostic framework aimed at identifying monogenic events that could explain the phenotype. The multifactorial nature of the disease requires a deeper exploration of patient genomes and case-control studies in large cohorts. However, in cases of syndromic forms or familial recurrences, pathogenic variants and known risk factors can be identified.

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P13.024.D From Case Reports to Comprehensive Understanding: Nearly a Decade of Witteveen-Kolk Syndrome

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Background: Witteveen-Kolk syndrome (WITKOS) is a rare neurodevelopmental disorder characterized by craniofacial dysmorphism, neurodevelopmental abnormalities, and short stature, caused by loss-of-function variants in the *SIN3A* gene. Since 2016, less than 80 cases have been described. This study focuses on three new Chilean cases and review of the 72 patients with *SIN3A* variants or CNVs known up to date.

Methodology: Detailed genetic analyses were performed for the Chilean cases, including whole exome sequencing and CNV analysis. A comprehensive literature review identified 13 publications with 69 previously reported cases, with data collected on craniofacial dysmorphisms, neurodevelopmental abnormalities and types of variants in *SIN3A* gene or CNVs that include this gene.

Results: Among the 72 patients, 76% were full-term newborns, 80% with normal birth weight. Developmental delay and intellectual disability were seen in 77% of cases, while 51% had psychiatric or behavioural issues, where autism spectrum disorder being the most common. Dysmorphic features are present in 72%, such as high forehead(63%) and elongated face(58%). Other findings are microcephaly, feeding difficulties, short stature, and low weight which were less frequent(less than 50%). Additional anomalies included CNS abnormalities(34%), skeletal(20%), cardiovascular(11%) and urogenital(7%). Most of the molecular findings were variants(76%), the majority of cases(78%) were classified as de novo.

Conclusion: Diagnosing WITKOS based on clinical presentation is challenging due to subtle facial features. The *SIN3A* gene's crucial role in cortical development may explain the syndrome manifestations. This study broadens the phenotype, understanding of WITKOS and emphasizes the importance of knowing its natural history for an effective long-term follow-up.

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P13.025.A Novel pathogenic mechanism in Rubinstein-Taybi Syndrome: alternative translation initiation in*CREBBP*

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Background: Rubinstein-Taybi syndrome (RTS) is a rare genetic disorder characterized by distinctive facial features, broad and often angulated thumbs and halluces, short stature, and moderate-to-severe intellectual disability. It is caused by loss of function variant or large deletions in ubiquitary expressed *CREBBP* (cAMP response element-binding protein-binding protein), which encodes a transcriptional coactivator critical for gene expression regulation through chromatin remodeling. Haploinsufficiency of *CREBBP* disrupts normal cell growth, differentiation, and survival. We describe the case of a young woman with a clinical presentation highly suggestive of RTS, in which the carrier status of a novel heterozygote, de novo variant in the 5'UTR of *CREBBP* was detected using whole genome sequencing. This variant creates a 195 bp *open reading frame* upstream to the physiologic translation initiation site that do not overlap with *CREBBP* protein sequence**. Hypothesis**: this variant generates a new competing initiating translation site which may lead to aberrant translation of a non-functional protein and disruption of normal *CREBBP* translation or transcription, fostering haploinsufficiency and RTS.

Material and Methods: we investigated our postulate through episignature analysis, protein and RNA expression assays, Ribo-sequencing, and in vitro translation assays.

Results: this study's findings reveal a new mechanism of pathogenicity associated with RTS and underscores the importance of translational regulation in *CREBBP* function.

Conclusion: our results highlight the necessity of whole genome sequencing to uncover elusive variants

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P13.026.B Familial recurrence of a Multilocus Imprinting Disturbance resulting in Beckwith-Wiedemann syndrome with Angelman features in a family with maternal NLRP2 mutation

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Background: Imprinting disorders are congenital diseases characterized by abnormal methylation patterns in differentially methylated regions. They are generally sporadic, but rare instances of familial recurrence raise suspicions of underlying contributing genetic factors. While these disorders typically involve single-locus defects, some cases exhibit methylation abnormalities across multiple loci, a phenomenon known as Multilocus Imprinting Disturbance (MLID), resulting in overlapping syndromes depending on the affected loci. Recurrent MLID is rare and may be due to maternal trans-acting genetic variants in genes involved in embryonic epigenetic reprogramming

Material and Methods: A patient was referred to our center with suspected Beckwith-Wiedemann Syndrome (BWS) with additional symptoms of severe neurodevelopmental delay with absent speech and behavioral disorder reminiscent of Angelman Syndrome (AS). His brother had been previously diagnosed with BWS. We performed methylation assays, genomic microarrays and trio (proband and parents) whole-genome sequencing.

Results: Methylation analysis identified widespread abnormal unbalanced methylation patterns across multiple loci, including the regions associated with Beckwith-Wiedemann and Angelman syndromes (KCNQ1OT1/11p15.5 and SNRPN/15q21). Trio-WGS did not reveal any sequence variant associated with neurodeveloppemental disorder in the proband but the homozygous NM_017852.5:c.1177del p.(Gln393Serfs*16) frameshift variant in maternal NLRP2.

Conclusion: Bi-allelic variants in NLRP2 have been reported in two families with MLID: one family with two children with BWS-MLID and delayed development, one family with a Silver-Russel-MLID. Six additional families with history of early embryonic arrest have also been recorded. We describe the third family with biallelic maternal NLRP2 variant resulting in recurrent MLID and Beckwith-Wiedemann and Angelman syndromes in the same patient.

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P13.027.C Genetic and Clinical Insights into Alazami Syndrome. a series of 22 patients

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Background: Alazami syndrome (AS) (MIM 615071) is a rare autosomal recessive disorder caused by pathogenic variants in LARP7, leading to severe intellectual disability, growth retardation, craniofacial dysmorphisms, and skeletal abnormalities. While the clinical spectrum of AS has been well described, the potential involvement of impaired telomere maintenance in the disease pathogenesis reported in two pedigrees, remains unclear. This study aims to expand the phenotype of AS and investigate the correlation between telomere length and clinical manifestations.

Materials and Methods: Following a multicentric collaborative initiative, we identified 22 individuals from 15 families with genetically confirmed AS. Clinical, radiographic, and molecular data were systematically collected. Telomere length were measured by quantitative PCR and flow cytometry-based methods.

Results: All individuals presented with severe intellectual disability, short stature, and characteristic craniofacial anomalies. Skeletal abnormalities, particularly delayed bone age, joint contractures, and scoliosis, were frequently observed. Additional features included feeding difficulties, recurrent respiratory infections, and genitourinary anomalies. Neurodevelopmental assessments revealed profound cognitive impairment with absent or very limited speech development. Behavioral abnormalities, including autistic traits and hyperactivity, were also reported. Telomere length analysis on blood performed in three individuals showed no significant shortening.

Conclusion: This study expands the phenotypic spectrum of AS and provides a comprehensive characterization of its clinical manifestations. The findings highlight the multisystem involvement in AS, emphasizing the importance of early diagnosis and multidisciplinary management. Although the number of cases analyzed is still limited, shortening of telomere length in blood cells has not been confirmed.

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P13.029.A Biallelic variants in *TMEM17* cause Meckel-Gruber syndrome within the ciliopathy spectrum

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Background: *TMEM17* encodes a transmembrane protein that is part of the cilium's transition zone; however, its exact function and structure remain unknown. Three cases have been published involving a primary ciliopathy phenotype associated with biallelic variants in *TMEM17*: NM_198276.2:c.306C>A p.(Asn102Lys) in two brothers with orofaciodigital syndrome and NM_198276:c.302G>T p.(Gly101Val) in a patient with Joubert syndrome. Here, further evidence for the role of the TMEM17 gene in cases of ciliopathies, including Meckel-Gruber syndrome (MGS) is provided.

Material and Methods: CENTOGENE Biodatabank containing information from over 800,000 individuals was queried for biallelic, rare variants in TMEM17 and related phenotypes. Human phenotypes are recorded as Human Phenotype Ontology terms. The gene-disease relationship (GDR) was evaluated according to the ClinGen recommendations.

Results: Four unrelated patients were identified in the Biodatabank with a diagnosis of MGS and novel homozygous variants in *TMEM17:* NM_198276.3:c4del p.(Glu2Serfs*58) in two cases, and NM_198276.3:c:366dup p.(Pro123Thrfs*9) and NM_198276.3:c.368C>G p.(Pro123Arg) in one case each. This allowed the upgrade of the GDR level of evidence from "limited" to "moderate". In contrast to the published patients, the newly identified cases presented prenatally with a very severe phenotype consisting of encephalocele, polycystic kidney dysplasia and polydactyly, with early lethality.

Conclusion: Biallelic variants in *TMEM17* are associated with ciliopathies, showing moderate evidence of a gene-disease relationship. This gene should be considered in the differential diagnosis of patients with a phenotype compatible with a primary ciliopathy. Additionally, the utility of bio-databanks in medical genetics is highlighted.

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P13.030.B Genetic and phenotypic spectrum of KBG syndrome: an Australian case series of 21 individuals

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Background: Heterozygous pathogenic loss of function *ANKRD11* variants or chromosome 16q24.3 deletions involving *ANKRD11* can cause KBG syndrome. This multi-system disorder has variable impacts on organ system development, learning, behaviour and growth. We describe a further 21 cases of KBG syndrome, not previously reported.

Material and Methods: Retrospective chart review was undertaken to document relevant clinical features and genetic testing results. Patient consent was obtained for publication of clinical photographs.

Results: 62% of the cohort were male. Age at time of diagnosis ranged from 0–20 years with a mean age of 8 years (SD = 5.2), including one antenatal case. 48% of genetic causes were determined to be de novo and 20% of cases were proven familial. 16q24.3 deletions with partial *ANKRD11* gene deletion were evident for 19% of the cohort, with one parent identified to be mosaic. Genetic variants included frameshift (48%), nonsense (24%), and missense (9%). Macrodontia, a triangular shaped face, synophrys, a distinctive nasal tip, long philtrum and thin upper lip were frequently reported. All patients had developmental delay, in particular speech delay. 52% had learning difficulties and 14% had verified intellectual impairment. 33% had hearing loss, 38% had musculoskeletal anomalies, 33% had short stature (<3rd centile), 33% had seizures and 24% had congenital heart disease. Novel findings for individual cases included anorectal malformation, bicornuate uterus and choanal atresia.

Conclusions: This case series demonstrates the wide spectrum of KBG syndrome and contributes further to the literature about the frequency of associated clinical features.

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P13.031.C Complete pseudoanodontia resulting from genomic rearrangement of the *SHH* locus

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Background: A mother-daughter pair presented with an unusual phenotype of mild developmental delay, distal arthrogryposis, exostosis of manubrium sterni, eruption failure of deciduous and adult teeth, and amblyopia. Family history negative. Chromosome analysis identified a de novo t(7;22)(q36.2;q13.32) balanced reciprocal translocation in both patients. We characterized the translocation with novel methods and identify a new phenotypic association with the *SHH* gene.

Material and Methods: Clinical laboratory genomics workup. Optical Genome Mapping (OGM), ONT long-read whole-genome sequencing (LRS) and RNA-seq. were performed.

Results: aCGH and short-read WGS were negative. LRS determined the precise breakpoint location (hg38 chr7:156,946,076 and chr22:45,309,078) and revealed a genomic rearrangement of the *SHH* locus. The *SHH* gene remains on derivative chr. 7 while its enhancers are translocated onto derivative chr. 22. The chr. 22 breakpoint is in the *FAM118A* gene, which has no known disease associations. RNA-seq. did not detect chimeric *FAM118A* fusion transcripts or abnormal expression of *FAM118A*. OGM did not detect any other structural variants that could explain their phenotype.

Conclusion: We elucidated the breakpoints of this de novo translocation in patients and identified a genomic rearrangement of the SHH locus. The rearrangement presumably results in disruption of the topologically associating domain of the *SHH* locus. This is the first reported case linking teeth eruption failure in humans to the *SHH* gene. This is supported by published mouse studies that showed total suppression of tooth eruption in neonatal mice following treatment with an Shh inhibitor. Hi-C pending.

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P13.032.D Genetic analysis in Rubinstein-Taybi syndrome

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Background: Rubinstein-Taybi syndrome (RTS) is characterized by distinctive facial appearance, broad thumbs, short stature, and intellectual disability. RTS is caused by heterozygous pathogenic variants or re-arrangements in the genes *CREBBP* and *EP300*, typically leading to haploinsufficiency. The diagnosis of RTS is made by the characteristic clinical findings and /or molecular genetic testing of a heterozygous pathogenic variant in *CREBBP* or *EP300*.

Material and Methods: Diagnostic approach of molecular genetic testing in RTS: Genomic DNA was extracted from peripheral blood. Targeted analysis of *CREBBP* and/or *EP300* was performed by Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) or array Comparative Genomic Hybridisation (aCGH).Whole-exome sequencing (WES), genome-wide methylation pattern (epigenetic signature) were also performed. Written informed consents were obtained from the parents of the patients in accordance with Institute Review Board and Ethics Committee.

Results: Ten patients with RTS were analyzed. Two nonsense variants, two frameshift variants, one splice donor variant, three small deletions, one small intragenic insertion/deletion in *CREBPP* and one nonsense variant in *EP300* were identified. The epigenetic signature was analyzed, as individuals with RTS showed a specific pattern.

Conclusion: RTS demonstrates significant phenotypic overlap with other Mendelian disorders, referred to as chromatinopathies, which affect chromatin structure on genome-wide scale. The identification of a pathogenic variant and genome-wide methylation pattern provides great support to confirm the clinical diagnosis.

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P13.033.A Novel deletion of *ATAD3* gene cluster as a cause of lethal pontocerebellar hypoplasia, hypotonia, and respiratory insufficiency syndrome

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Background: Neonatal lethal pontocerebellar hypoplasia, hypotonia, and respiratory insufficiency syndrome (PHRINL) is an autosomal recessive multisystem disorder with onset in utero and death in the neonatal period. We have found a homozygous deletion involving exons 6–16 of the *ATAD3B* gene and exons 1–2 of the *ATAD3A* gene in a newborn who died after birth and whose mother was pregnant at the time of the analysis.

Methods: We performed massive parallel sequencing on the NextSeq550 Illumina platform using the Clinical Exome Solutions v3 and Whole Exome Solutions v2 gene panels from Sophia Genetics.

Results: The full-term newborn was severely impaired in consciousness, unresponsive to painful stimuli, unable to open eyes, had hypertonicity, hypotrophy, encephalopathy, sepsis, pneumonia, respiratory failure, heart failure, petechiae, coagulopathy, cryptorchidism. We performed exome sequencing on a deceased newborn, mother, father, and amniotic fluid from an ongoing pregnancy. Deletions in the *ATAD3* gene cluster were detected in this family. The father has a heterozygous deletion including exons 4–16 of the *ATAD3B* gene and exons 1–4 of the *ATAD3A* gene and the mother has a heterozygous deletion including exons 10–12 of the *ATAD3C* gene, the entire *ATAD3B* gene (exons 1–16) and exons 1–2 of the *ATAD3A* gene. In the current pregnancy, the maternal variant was inherited and the second child is a healthy carrier.

Conclusion: We were able to identify new, previously undescribed deletions in the *ATAD3* gene cluster that were present in both parents and whose overlap was the cause of the homozygous deletion in the first deceased offspring.

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P13.034.B In-vitro targeting of esophageal atresia candidate pathways with small molecules to investigate rare variant burden

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Background: Esophageal atresia (EA) and tracheoesophageal fistula (TEF) arise after defective tracheoesophageal separation. Half of the patients have additional anomalies. Parents are usually unaffected, making de novo or recessive DNA alterations a plausible explanation. Patients with a more complex phenotype (including VACTERL) are known to be enriched for de novo variants. We hypothesize that part of the missing heritability is due to this subgroup of patients. Moreover, a substantial locus heterogeneity contributes to the low diagnostic yield. Our prior studies indicate disrupted actin cytoskeleton organization, BMP-signaling, disrupted cell adhesion, motility, and migration in TEF. We aim to investigate rare variants impacting these pathways by inhibiting and stimulating patient fibroblasts with small molecules.

Material and Methods: We selected twenty complex EA/TEF patients for short- and long-read genome sequencing, of which some have also been subjected to RNA-sequencing. We examine differences in transcriptomes, DNA damage response and cell morphology and behavior in patient versus control fibroblasts. Subsequently, we evaluate the contribution of deleterious de novo and inherited genetic changes in identified affected pathways.

Results: We identified several rare inherited variants in candidate pathway genes. We have determined IC50 values of small molecules in control fibroblasts and are currently investigating the differences of inhibiting and stimulating candidate pathways on patient vs control transcriptomes.

Conclusion: Further evaluation of pathway alteration in vitro should highlight the effect of the variants found. Combined, these results and a pathway-focused approach may improve our understanding of EA/TEF etiology and increase the diagnostic yield of NGS based approaches.

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P13.035.C Phenotypic spectrum of Miller syndrome: insight from a french cohort

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Background: Miller syndrome (MIM #263750) is a rare autosomal recessive acrofacial dysostosis caused by biallelic variants in *DHODH*, a key enzyme in pyrimidine biosynthesis. Since its identification in 2010, case reports have highlighted its variable phenotype, emphasizing the need to refine the clinical spectrum.

Material and Methods: We present a cohort of ten individuals from seven families, aged from prenatal diagnosis to 46 years, identified through national collaboration. Molecular analyses were performed via gene panel, exome, or genome sequencing. Clinical data were collected through standardized forms during routine care.

Results: This is the largest Miller syndrome cohort to date, revealing a broad phenotypic spectrum. We describe a novel finding of optic atrophy in multiple members of one consanguineous family. Typical postaxial limb defects, including 5th digit absence, were consistent with prior reports, but we also noted thumb and tibial hypoplasia, suggesting broader preaxial involvement. A higher prevalence of camptodactyly and facial hemangiomas in two patients were notable findings. Congenital heart defects, mainly atrial septal defects, were common, while neurodevelopment remained normal in all living individuals.

Conclusion: Our findings expand the Miller syndrome phenotype, particularly regarding preaxial limb anomalies and facial hemangiomas. The optic atrophy observed in one family warrants further screening to assess its association with the syndrome. Early prenatal diagnosis, especially in cases with limb and cardiac malformations, is crucial for management and genetic counseling.

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P13.036.D Unravelling the molecular basis of Xia-Gibbs syndrome

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Background: Xia-Gibbs syndrome (XIGIS; OMIM #615829) is a rare neurodevelopmental disorder, characterised by various physical and neurological features, including cognitive impairment, motor delays, mild dysmorphism, and autism spectrum traits. XIGIS is associated with heterozygous mutations in the *AT-Hook DNA Binding Motif Containing 1* (*AHDC1*) gene, though the pathogenic mechanisms remain unclear. Preliminary evidence suggests that AHDC1 is involved in transcriptional regulation during differentiation and retinoic acid response.

Material and Methods: To investigate the effects of *AHDC1* mutations, we established lymphoblastoid cell lines in collaboration with the Italian Xia-Gibbs Society. After confirming the *AHDC1* expression, we performed polyA RNA-sequencing of three families to identify differentially expressed genes (DEGs) in the probands compared with their parents. Additionally, we developed a custom antibody targeting the AHDC1 N-terminal region to detect wild-type and potential truncated mutant protein forms via western blot (WB).

Results: RNA sequencing revealed several DEGs in the probands, involved in RNA processing and extracellular organisation. No significant reduction in *AHDC1* mRNA levels was observed in the probands compared with their parents, and mutant transcripts were detected in all XIGIS subjects. Additionally, the custom antibody was validated, and analysis of truncated AHDC1 forms is ongoing.

Conclusion: Our initial findings suggest that *AHDC1* mutations may lead to a gain-of-function mechanism. The identification of DEGs provides insight into potential molecular pathways involved in XIGIS. The successful validation of the custom antibody paves the way for detecting truncated AHDC1 protein forms, further refining our understanding of the molecular mechanisms underlying the syndrome.

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P13.037.A Dual diagnosis of two overgrowth conditions in the same individual: Sotos and Tatton-Brown-Rahman syndromes. What is the cancer risk?

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Material and Methods: We report on a 10-year-old female, third-born to Moroccan consanguineous (first cousin) parents. She was evaluated for classic medulloblastoma (diagnosed at 5 years of age), chronic renal insufficiency, CMV-blindness, and panhypopituitarism. She presented with macrosomia, advanced bone age and joint hyperlaxity with motor impairment.

Results: Trio whole exome sequencing (WES) identified two heterozygous de novo variants: c.6455G>A,p.(Arg2152Gln) in *NSD1* and c.2644C>T,p.(Arg882Cys) in *DNMT3A*, both classified as pathogenic (ACMG).

Previous genetic analyses (karyotype, array-CGH and methylation study of the 11p15.5 region) were normal.

Conclusion: We present a dual diagnosis of two distinct de novo overgrowth conditions in a patient born to consanguineous parents. The co-occurrence of two genetic disorders is a growing phenomenon with the advent of next-generation technologies.Our patient shows variable features of both Sotos and Tatton-Brown-Rahman (TBRS) syndromes, as well as some distinctive facial features reminiscent of Sotos (evident in the childhood photos shown by the family at first genetic evaluation) before the cancer diagnosis and treatment. Furthermore, our case adds some novel features that include pericardiac effusion, which has only been reported in another patient with TBRS. Given the patient's history, the oncological risk associated with both syndromes and the lack of consensus guidelines regarding cancer screening for both conditions, this dual diagnosis represents a new challenge for healthcare professionals in terms of planning personalised surveillance.

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P13.038.B Two novel pathogenetic variants caused TLK2 induced neurodevelopmental disorder associated to craniosynostosis in one

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Background: *TLK2* is a gene encoding a protein forming homo- and hetero-dimers involved in DNA replication,cell cycle checkpoint recovery and chromatin remodeling.It is responsible for a neurodevelopmental disorder(NDD)(#618050) characterized by developmental delay(DD)/intellectual disability(ID),behavioral issues,microcephaly,gastro-intestinal problems and dysmorphisms.

Material and Methods: Here, we report two children with two different de novo, not yet reported, pathogenic variants (PVs) in *TLK2*:c.878delinsAAGC;p.Arg293delinsGlnAla and c.2117_2118dup;p.Arg707Thrfs*103 (NM_006852).They both had microcephaly,short stature,DD,hypotonia and dysmorphisms.One child also presented IUGR,monocoronal synostosis,joint hypermobility and constipation.We also reviewed literature debating TLK2-NDD clinical and molecular characteristics.

Results: Forty-three other patients were identified.The features of all known TLK2 children are summarized in table. Penetrance is complete, the phenotype variable. Only one homozygous patient (c.163 A>G; p.Lys55Glu) has been reported with a more severe phenotype and healthy carriers' parents.The forty-four PVs reported were distributed along the entire gene.Truncating variants were 38,6%, missense and splicing 29,5% each,and indels 2,3%.

Conclusions: We confirm that the key features of TLK2-NDD are DD/ID, microcephaly, hypotonia, short stature and behavioral issues.Yet *TLK2* can be even a rare cause of syndromic craniosynostosis.Given the extreme rarity of the disorder,the lack of hotspot PVs, the definition of genotype-phenotype correlation is still an object of research.Even haploinsufficiency seems to be the most likely pathogenetic mechanism, dominant negative effect for at least some missense variants has been suggested.Regulation and dimeric structure of *TLK2* warrant further investigation.Description of additional cases, the parallels with other overlapping genetic disorders due to chromatin regulation might offer insights for further studies.

	Affected/Total	%
Speech delay/Developmental delay	42/45	93
Intellectual disability	36/45	80
Facial anomalies	34/45	75.5
Learning disabilities	32/45	71
Constipation	23/44	52
ADHD	19/45	42
Autism and behavioural disorder	17/45	38
Hypotonia	16/44	36
Refraction anomalies	13/44	29.5
Microcephaly	11/43	25.5
Short stature	18/44	24
Recurrent otitis media	10/45	22
Joint hypermobility	9/43	21
Epilepsy	6/42	14
Craniosynostosis	5/45	11
Scoliosis	4/43	9

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P13.039.C Further clinical delineation of a *MED13L* recurrent missense variant at codon Pro866 expands the *MED13L*-related syndrome beyond haploinsufficiency

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Background: *MED13L* encodes a subunit of the CDK8 kinase module of the Mediator complex. *MED13L* haploinsufficiency leads to syndromic intellectual disability (ID) with minimal or absent speech, hypotonia, behavioral symptoms, seizures and musculoskeletal malformations, with facial features including upslanted and/or short palpebral fissures, broad forehead and large open mouth appearance. Several missense variants have been reported, some of which are recurrent and clustered in exons 15–17 and 25–31. Clinical details and photographs are available for one individual reported with the p.Pro866Leu variant and three with the p.Pro869Ser variant (both within exon 15). These individuals are more severely affected than those with truncating variants.

Methods/Results: Here, we report three unrelated individuals harbouring the recurrent de novo p.Pro866Leu variant and sharing a strikingly similar presentation including pre- and/or post-natal progressive microcephaly, moderate to severe ID, poor/absent speech, hyperlaxity, brachytelephalangy of fingers with fetal pads, severe scoliosis, coxa valga, gracile bones, osteoporosis and fractures. Facial features include stiff skin with a facial mask appearance, metopic ridge, long and everted lower eyelids and thin upper lip. Interestingly, previously reported individuals with the p.Pro866Leu and p.Pro869Ser variants share the same gestalt, in contrast to patients presenting truncating variants. Microcephaly and slender habitus are at odds with the frequent stocky habitus of individuals with classic *MED13L*-related syndrome.

Conclusion: These three cases strengthen the hypothesis of dominant-negative or gain-of-function mechanism for this cluster of missense variants affecting highly conserved residues (Pro866 and Pro869) and leading to a novel and recognizable clinical presentation.

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P13.041.A WGS identifies compound heterozygous deep-intronic variant and complex rearrangement in VPS13B in a patient with Cohen syndrome

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Background/Results: Recently, we implemented diagnostic whole genome sequencing (WGS) to replace exome sequencing. For a patient with high myopia, rod-cone dystrophy, mild cataract, intellectual disability, short stature, microcephaly, central obesity, small hands and feet, hyperlaxity and dysmorphic features this allowed us after ten years to obtain a diagnosis. The family history was negative for vision loss problems, but her brother had intellectual disability and Duchenne muscular dystrophy due to a maternal *DMD* deletion, which the patient did not inherit. Exome analysis with a vision-loss panel did not identify abnormalities, so we expanded the search with a broad gene panel (clingenV1). Two variants were detected in the *VPS13B* gene: a maternal complex rearrangement causing a partial exon 14 deletion, and a deep intronic variant (NM_017890.5(VPS13B):c.4042+258C>G) on the paternal allele, predicted to strengthen a deep-intronic cryptic splice site. Subsequent analysis on RNA from blood cells using RT-PCR and Sanger sequencing showed the inclusion of a pseudo-exon, leading to a predicted frameshift, and nonsense-mediated mRNA decay: NM_017890.5(VPS13B):r.4042_4043ins4042+172_4042+257, p.(Glu1348Glyfs*2). Together, this confirmed the diagnosis Cohen syndrome. Subsequent analysis of 10 WGS datasets revealed 5–21 rare deep-intronic variants per patient (average 11.8), with specific panels reducing this to 0–6 (average 2.1), suggesting the feasibility of analysis of deep-intronic variants.

Conclusion: In this case the availability of WGS data showed the superiority in detecting structural variants and the capacity to directly prioritize relevant deep-intronic variants.

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P13.042.B Expanding the PTEN Mutational Spectrum: Identification of Eight Novel Variants and Genotype-Phenotype Correlations in a Multicenter PHTS Cohort

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Background: PTEN Hamartoma Tumor Syndrome (PHTS) is an autosomal dominant disorder characterized by macrocephaly, neurodevelopmental abnormalities, hamartomatous growths, and increased cancer risk. Despite its clinical heterogeneity, genotype-phenotype correlations remain poorly understood. This study investigates PTEN mutational profiles and associated clinical features in a multicenter cohort, expanding the mutational

spectrum with the identification of 8 novel variants.

Methods: Thirty-eight patients were recruited from Cervello Hospital (Palermo) and IRCCS Giannina Gaslini (Genoa). Genetic sequencing identified 29 PTEN variants, classified as pathogenic (P) or likely pathogenic (LP) according to ACMG guidelines. Clinical, neuroimaging, and gastrointestinal data were systematically reviewed to assess phenotypic variability and age-related manifestations.

Results: A total of 29 distinct variants were identified (14 missense, 8 nonsense, 4 frameshift, 3 splice-site), including 8 novel mutations (3 missense, 1 nonsense, 3 frameshift, 1 splice-site). Macrocephaly was present in 89.4% of cases, frequently associated with neurodevelopmental disorders (42.1%) and autism spectrum disorder. Thyroid pathology was observed in 26.3%, including carcinoma in 3 patients. Gastrointestinal polyposis was found in 15.8%, with mixed and hamartomatous histology. Neuroimaging abnormalities were detected in 31.6%, including hydrocephalus (13.2%) and Chiari I malformation (7.9%). Missense mutations were predominantly associated with neurodevelopmental disorders, while truncating mutations showed unexpected links to these phenotypes, suggesting a broader PTEN-related neurodevelopmental impact.

Conclusion: This study expands the PTEN mutational spectrum with 8 novel variants, refining genotype-phenotype correlations and highlighting the need for early genetic diagnosis and personalized follow-up strategies. A multidisciplinary approach is essential to optimize clinical management and long-term surveillance in PHTS patients.

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P13.043.C A clinical study of Japanese patients with ReNU syndrome

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Background: ReNU syndrome, also known as neurodevelopmental disorder with hypotonia, brain anomalies, distinctive facies, and absent language (NEDHAFA), is characterized by hypotonia, global developmental delay, intellectual disability (ID) with poor or absent speech, delayed motor development, feeding difficulties, short stature, seizures, and dysmorphic features. Neuroradiological abnormalities including ventriculomegaly, hypoplasia of corpus callosum, and decreased white matter volume are observed. Most of the individuals have the same highly recurrent single base insertion (n.64_65insT) in *RNU4-2*. *RNU4-2* encodes the U4 small nuclear RNA (snRNA), which is a critical component of the U4/U6.U5 tri-snRNP complex of the major spliceosome.

Material and Methods: We reviewed patients with neurodevelopmental disorders that matched the clinical features of ReNU syndrome and performed hotspot analysis using Sanger method. Whole-exome sequencing did not identify any variants that could explain the symptoms in all cases. A recurrent variant was identified in 6 cases, while a novel variant, n.66A>G, was detected in one case.

Results: The 7 patients were aged between 4 and 27 years. All patients showed severe developmental delay and/or ID. Independent walking was achieved in 4 cases. In 5 cases, meaningful words had not been acquired even after the age of 5 years. All patients showed a distinctive pattern of dysmorphic features, including hooded upper eyelid, full cheeks, tented philtrum, and mouth constantly open with an everted lower lip vermilion. All patients showed neuroradiological abnormalities.

Conclusion: The identification of 7 patients at a single institution reaffirmed that ReNU syndrome is an important cause of ID. We identified a novel variant in *RNU4-2*.

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P13.044.D Pile on many more layers: a case of neurodevelopmental and hormonal disorder illustrating the complexity of multiple diagnoses

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Background: About 10% of cases with a complex rare condition are actually expected to have multiple Mendelian diseases. Ascertainment can be hindered by their overlapping effects, as illustrated by the triple diagnosis in this case report.

Material and Methods: Imaging, laboratory tests and specialistic evaluation according to good clinical practice; WES (SNV +CNV analysis) filtered for neurodevelopmental disorders.

Results: The proband, a 12 year old boy, is the third child of healthy nonconsanguineous parents. At age 1 year, trigonocephaly due to early closure of the metopic suture was detected. During childhood he showed language delay and was diagnosed with mild to moderate intellectual disability. At age 9 years premature pubarche and high levels of 17-hydroxyprogesterone were noted, and *CYP21A2* gene testing confirmed homozygous pathogenic variant NM_000500.9:c.[844G>T];[844G>T], p.[Val282Leu];[Val282Leu], associated with late-onset adrenal hyperplasia syndrome (NC-CAH). At age 10 years onset of epilepsy with focal impaired awareness seizures prompted neuropsychiatric evaluation at our hospital and WES analysis, which detected a 47,XXY karyotype and a de novo variant in the *ZMYM2* gene: NM_003453.6:c.[3976del];[=], p.[(Tyr1326Ilefs*35)];[(=)] (ACMG/AMP class 4).

Conclusion: NC-CAH may explain the trigonocephaly and apparently offsets the hypogonadism associated with Klinefelter syndrome; a follow-up throughout puberty will elucidate this effect. The presentation is complicated by a neurodevelopmental disorder likely caused by a hypomorphic frameshift alteration of transcriptional regulator *ZMYM2*. Recognition of the multiple diagnosis will aid in addressing each aspect appropriately.

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P13.045.A Mosaic *KMT2D* variant in Kabuki syndrome - importance of DNA methylation profiling in diagnostics

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Background: Pathogenic variants in *KMT2D* gene cause autosomal dominant Kabuki syndrome (KS), a rare congenital neurodevelopmental disorder characterized by intellectual disability, visceral malformations, persistence of fetal fingertip pads, short stature, skeletal anomalies and combination of specific facial features. In recent years more than 11 individuals with mosaic pathogenic *KMT2D* variants have been reported, with variant allele frequency (VAF) 10%–37%, suggesting that the presence of the variant even in just mosaic form can lead to an almost fully penetrant phenotype. In addition, a distinctive and similar epigenetic signature has been identified in blood of both mosaic and heterozygous patients, proposing the DNA methylation profiling as a diagnostic tool for better interpretation of *KMT2D* variants.

Material and Methods: A four-year old male with hypotonia, unilateral renal agenesis, short stature, downslanted palpebral fissures, microcephaly, premature birth, motor delay, and mild global developmental delay was referred to singleton whole exome sequencing (WES).

Results: WES and subsequent Sanger sequencing analysis revealed a nonsense variant c.8311C>T p.(Arg2771*) in the *KMT2D* gene in mosaic form (VAF 12–19%). This variant has been previously reported in KS patients, but only in heterozygous form. Methylation studies are ongoing.

Conclusion: We report a rare case of *KMT2D* mosaic variant leading to Kabuki syndrome. It highlights the need for variant testing in alternative tissues as well as DNA methylation profiling to better interpret WES results, especially for Mendelian disorders caused by defect in epigenetic machinery.

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P13.046.B Molecular and phenotypic characterization of 21 Polish patients with *CREBBP/EP300* mutations: from Rubinstein-Taybi to Menke-Hennekam syndromes

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Background: Rubinstein-Taybi syndrome (RSTS) is characterized by distinctive facial dysmorphia, broad thumbs/halluces, short stature, hearing loss, heart/renal defect, cryptorchidism and intellectual disability. RSTS is associated with heterozygous mutations in *CREBBP* or *EP300* genes, with expected detection rates of 88% for *CREBBP* SNV, 12% for *CREBBP* CNV, and 8–10% for *EP300* mutations. Variants in 30–31 exons of *CREBBP* or in the homologous regions of *EP300* are associated with Menke-Hennekam syndrome, characterized by developmental delay, distinctive facial features, short stature and behavioral problems.

Material and Methods: The study included 21 patients with a clinical suspicion of either RSTS or an unspecified neurodevelopmental-dysmorphic syndrome (in line with the 'from genotype to phenotype' approach in case of the latter), diagnosed through comprehensive genetic testing (NGS and MLPA/aCGH, where applicable).

Results: Molecular analysis revealed mutations in *CREBBP* (15 patients, 71%) and *EP300* (6 patients, 28%), including frameshift indels(24%), missense(29%) / nonsense(14%) / splice site(5%) variants, or larger deletions(24%). The distribution of variant types shows slightly higher proportion of deletions compared with expected rates. Remarkably, 48% of identified variants were novel (*CREBBP*: c.-49_12del, c.289C>T, c.1094A>G, c.4133+2T>G, c.5028_5029dup, c.7174G>A; *EP300*: c.1093_1096del, c.3044_3045del, c.3205G>A, c.4713_4722del), with 8/10 occurred de novo. Most patients displayed typical RSTS phenotype. Notably, two patients with a *CREBBP* variant in exon 30(c.5028_5029dup) or 31(c.5614A>G) presented clinical features distinct of RSTS, but consistent with Menke-Hennekam syndrome.

Conclusion: This cohort significantly expands the known mutation spectrum of RSTS and highlights the importance of careful genotype-phenotype correlation in patients with variants in exons 30–31 of *CREBBP*.

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P13.047.C Mosaic*KMT2D*Mutation in a Patient with Subtle Kabuki Syndrome Facial Dysmorphisms, Aortic Carctation and Mild Neurobehavioral Features: Case Report and Review of Clinical and Molecular data

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Background: Kabuki syndrome (KS) is a rare genetic disorder characterized by multiple congenital anomalies, variable intellectual disability and distinct facial features. Most cases are caused by variants in KMT2D and, less frequently, in KDM6A. Up to now, mosaic KMT2D variations have been documented in 12 individuals with allele frequencies ranging from 10% to 37% in blood cells.

Material and Methods: We report on a 7-year-old boy clinically suspected for KS because of mild facial features including elongated palpebral fissures, sparse lateral eyebrows, large anteverted ears, prominent fetal fingertip pads, fifth digit brachymesophalangy. Stature was in the low-normal range and he presented congenital heart disease (CHD) including aorta coarctation, surgically corrected at 2 months, bicuspid aortic valve, parachute mitral valve dysplasia. He also showed neurodevelopmental delay (speech delay, hyperactivity).

Results: Next-generation sequencing (NGS) panel including KMT2D, KDM6A, RAP1A, RAP1B and HNRNPK genes identified a previously reported KMT2D nonsense variant resulting in a premature stop codon (p.Arg5501Ter) at a low allele fraction of 20% in peripheral blood as the molecular defect underlying the trait.Analysis of available records of *KMT2D* mosaic cases was performed to outline the possible distinguishing features in comparison with the germline KS clinical spectrum. The majority of these patients display milder craniofacial and growth phenotypes and do not have CHD.

Conclusion: Consistent with previous data, we provide evidence that mosaic *KMT2D* mutations may result in subtle KS facial dysmorphisms and mild neurobehavioral features. Additionally, our report emphasizes that tipycal CHD (including aorta coarctation) of constituional KS can also be present in a low rate mosaicism.

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P13.048.D Beyond a single syndrome: the importance of comprehensive genetic analysis in complex cases

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Background: Genetic disorders often present with complex and overlapping phenotypes, making accurate diagnosis challenging. The assumption that all symptoms arise from a single syndrome can lead to misdiagnosis or incomplete understanding of a patient's condition. We present the case of a 2-month-old female infant born via in vitro fertilization, complicated by maternal hypertension, and delivered at term with an optimal APGAR score and a birth weight of 3010 g. Postnatally, she exhibited skin blistering, nail loss, and mucosal involvement. Due to developmental delay and suspected seizures, further investigations were conducted, leading to the identification of two distinct genetic disorders—Epidermolysis Bullosa (EB) and Beta-Ureidopropionase Deficiency (BUPD).

Material and Methods: A whole exome sequencing with a panel for EB was performed, and reanalysis of the initial NGS data was conducted due to additional symptoms.

Results: An NGS panel identified a homozygous pathogenic variant in LAMB3 (c.727C>T, p.Gln243Ter), confirming Junctional EB, which explained the dermatological manifestations. Subsequent reanalysis revealed a homozygous likely pathogenic variant in UPB1 (c.917-1G>A), associated with Beta-Ureidopropionase Deficiency, explaining the neurological symptoms. This case underscores the importance of considering multiple genetic diagnoses when clinical findings do not align with a single disorder.

Conclusion: Clinicians must remain vigilant to the possibility of dual diagnoses rather than attributing all symptoms to one condition. While targeted panel testing is cost-effective and time-efficient, it may overlook pathogenic variants in genes not included in the panel. Comprehensive genomic analysis should be considered in complex cases, supporting ACMG guidelines advocating for reanalysis when clinical suspicion persists.

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P13.050.B A de novo *MYRF* pathogenetic variant in a patient with Cornelia de Lange syndrome

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Background: Cornelia de Lange syndrome (CdLS) is a rare neurodevelopmental disorder characterized by peculiar features and caused by variants in genes encoding for components of cohesin complex or related proteins (OMIM #122470, #300590, #610759, #614701, #300882, #620568). These genes account for 75–80% of patients, whether some patients remain without molecular diagnosis and several other genes have proposed as causative.

Material and Methods: A patient with fetal diaphragmatic hernia was referred to our prenatal diagnosis clinic and karyotype and SNP array were performed. After birth, targeted gene sequencing and MLPA on patient's sample and trio-whole exome sequencing (WES) were carried out. Bioinformatic analyses were performed exploiting databases for assessing pathogenicity of the variant. Then, protein prediction tools were used for protein interactions analysis.

Results: The newborn had no vital signs at birth, and he showed clinical features of classic CdLS (clinical score evaluation > 11). A de novo frameshift variant in *MYRF* was identified by trio WES: c.1276delC, p.(Leu426Serfs*6). Our patient showed overlapping features with Cardiac-urogenital syndrome (CUGS, OMIM# 618280), caused by de novo variants in *MYRF*, encoding for a transcription factor which regulates CNS myelination and embryonic development. In addition, pathogenicity of the identified variant was sustained by protein prediction tools which show MYRF disruption and a correlation with members of the cohesin complex causative of CdLS was suggested by predicted protein interactors.

Conclusion: These findings expand CdLS molecular range and suggest a possible role for MYRF in this context. Its molecular interactions in genetic disorders should be further addressed.

C.O.I. Disclosure:

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P13.051.C Unraveling a blended phenotype through reverse phenotyping, a case of prolidase deficiency and epiphyseal dysplasia

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Background: A 25-year-old patient with chronic, painful skin ulcers on the lower extremities and epiphyseal dysplasia presented to our clinic. The patient had been experiencing skin lesions for 10 years and was previously diagnosed with vasculitis. However, the lesions remained unresponsive to treatments, including prednisone, colchicine, and cyclosporine. Additionally, the patient had bilateral avascular necrosis, with a history of left hip surgery. After being advised to undergo right hip surgery, but with persistent skin lesions, the patient was referred to our clinic by the dermatology department for further evaluation.

Material and Methods: Genomic DNA was extracted from a peripheral blood sample of the patient for clinical exome analysis. Exons and exon-intron transition regions of 4490 genes were investigated by next-generation sequencing. Variants were analyzed using the Genomize SEQ platform.

Results: A homozygous pathogenic variant of *PEPD* (NM_000285.4) c.549-1G>A, and a heterozygous likely pathogenic variant, *COMP* (NM_000095.3) c.819C>A (p.Asp273Glu), were observed in the patient's genomic data, and were correlating with the patient's clinical findings.

Conclusion: This case demonstrates the value of reverse phenotyping in clarifying a blended phenotype resulting from two distinct genetic disorders. The identification of a homozygous pathogenic *PEPD* variant redefined the patient's treatment-resistant skin ulcers as prolidase deficiency, rather than vasculitis. Additionally, the heterozygous *COMP* variant explained the musculoskeletal complications, including epiphyseal dysplasia and bilateral avascular necrosis. By integrating genomic and clinical data, reverse phenotyping enabled a more accurate diagnosis, highlighting the importance of comprehensive genetic analysis in cases with atypical or overlapping phenotypes, ultimately improving patient management.

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A. Peker: None. **A. Aykut:** None. **I. Karaarslan:** None. **H. Akın:** None. **A. Durmaz:** None.

P13.052.D Expanding the phenotype: long-QT associated with *EMC1* compound heterozigosity

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Background: Biallelic pathogenic variants at *EMC1* are associated with "Cerebellar atrophy, visual impairment, and psychomotor retardation" (MIM #616875), an ultrarare autosomal recessive neurodegenerative disorder with neonatal presentation of hypotonia, other neurological features, brain abnormalities (namely thin *corpus callosum* and cerebellar atrophy), and craniofacial dysmorphisms.

Methods: Clinical description of severe unusual presentation of *EMC1*-related disorder.

Results: We describe a male infant, born to healthy non-consanguineous parents, with prenatal presentation of sinus bradycardia and premature atrial contractions. He was admitted at birth in the Neonatal Intensive Care Unit with respiratory distress and hypotonia. This infant was subsequently diagnosed with long-QT and dilated cardiomyopathy with progressive heart failure. He maintained progressive global developmental delay, with poor eye contact, severe hypotonia, and facial hypomimia. Seizures were suspected at 5-months-old (right oculocephalic version and bilateral arm flexion), with EEG disclosing bilateral temporo-parietal interictal epileptiform discharges and an abnormal background. Brain imaging showed mega cisterna magna and a small *corpus callosum*. The boy had sudden death at 7.5 months of age, likely due to long-QT-related dysrhythmia. Trio Whole Exome Sequencing identified two compound heterozygous variants in *EMC1* gene, classified as likely pathogenic.

Conclusion: To the best of our knowledge, biallelic *EMC1* pathogenic variants are rarely associated with long-QT and neurodevelopmental delay with seizures. Recently, a case report of two infant siblings linked a similar cardiac phenotype with compound heterozygosity in *EMC1*. These findings show the importance of expanding the current phenotypic knowledge on *EMC1*-related disorders, possibly associating high cardiac risk due to potentially lethal QT-long-related arrythmias.

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P13.053.A Atypical presentation of Beckwith-Wiedemann syndrome revealed coexistence of Snijders Blok-Fisher syndrome in a 4-year-old male patient

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Background: Snijders Blok-Fisher syndrome (SBF, OMIM #618604) is a rare, autosomal dominant neurodevelopmental disorder characterized by mild-severe intellectual disability, dysmorphism, hypotonia, seizures, and central nervous system abnormalities. The clinical presentation is variable in severity and is associated with de novo pathogenic variants in *POU3F3* gene. Beckwith-Wiedemann syndrome (BWS, OMIM #130650) is a rare imprinting disorder characterized by macrosomia, macroglossia, hemihypertrophy, abdominal wall defects, and predisposition to embryonal tumors. BWS is primarily caused by alterations in the imprinted gene cluster on chromosome 11p15.5. This imprint locus consists of imprinting center 1 (IC1) that regulates the expression of IGF2 and H19 genes and imprinting center 2 (IC2) regulates the expression *of CDKN1C, KCNQ10T1*, and*KCNQ1* genes.

Results: Here we report a 4-year-old male patient who was referred to our outpatient clinics for macroglossia, neurodevelopmental delay and seizures. MS-MLPA analysis was done initially to evaluate methylation status of IC1/IC2 locus and hypomethylation of IC2 was detected. Since the patient had neurodevelopmental delay and IC2 hypomethylation, additional MS-MLPA analyses of *PLAGL1* and *SNRPN/UBE3A* locus were performed to exclude multilocus imprinting defects, and the results were normal. Additionally, whole-exome sequencing was done and a de novo pathogenic variant was detected at *POU3F3* gene.

Conclusion: To our knowledge, this is the first reported case of a dual diagnosis of Beckwith-Wiedemann and Snijders-Blok-Fisher syndromes in a patient. In cases where clinical findings are inconsistent with the primary diagnosis, it is essential to consider the possibility of concurrent involvement of two relatively common or rare inherited genetic conditions.

C.O.I. Disclosure:

S. Kocagil: None. **E. Akbaş:** None. **O. Çilingir:** None.

P13.054.B The benefit of staying active in a foreign environment

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Background: In X-autosome translocations, the spread of X-inactivation plays a critical role in determining the expression of autosomal genes. In addition, the parental origin of a de novo deletion may also have clinical significance. Our aim was to identify the etiology of severe hypotonia, cleft palate, macrocephaly, craniosynostosis, corpus callosum dysgenesis and cerebral atrophy in a female newborn.

Material and Methods: G-banded karyotyping, fluorescence in situ hybridization, array comparative genomic hybridization (aCGH), haplotype analysis and Sanger sequencing of heterozygous coding variants (rs2899642; rs3027875) in *RNF111* (15q22.1) and *HCFC1* (Xq28) genes in PBMC derived cDNA were performed.

Results: The karyotype was 45, X, der(X) t(X;15)(q28;q15),-15. The X chromosome involved in the translocation was intact, but a 10-Mb region on chromosome 15 with breakpoints at 15q11.2q13.3 containing the Prader-Willi/Angelman critical region was deleted. The deletion was paternal in origin. The cDNA Sanger sequencing of rs2899642 (*RNF111*, 15q22.1) and rs3027875 (*HCFC1*, Xq28) variants revealed skewed X-inactivation: 75–80% of cells expressed the involved X and both distal 15q regions.

Conclusion: The child's symptoms were attributed to the paternal 15q11.2q13.3 deletion, larger than typical in Prader-Willi syndrome. While in unbalanced X-autosome translocations there is a preferential silencing of the abnormal X, a skewed inactivation of the normal X allowed here the expression of the distal 15q22.1 region, consistent with the survival advantage. <!--EndFragment-->The phenotype of the child was thus strongly influenced by both the parental origin of the translocation and the skewed X-inactivation pattern.

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Z. Némethi: None. **E.G. Jávorszky:** None. **Á.F. Kovács:** None. **I. Haltrich:** None. **K. Tory:** None.

P13.055.C Clinical and anthropometric characterization of 100 brazilian patients with 22q11.2 deletion syndrome

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Background: The 22q11.2 deletion syndrome is a multisystem genetic disorder resulting from a hemizygous microdeletion, with a prevalence of approximately 1:4,000 live births. This study provides a comprehensive clinical and anthropometric profile of Brazilian patients, offering insights into the phenotypic diversity and health challenges associated with this condition.

Material and Methods: We conducted a retrospective review of medical records spanning 2000–2024 from a single specialized genetic center in Brazil. Clinical data, confirmed primarily via MLPA and occasionally by FISH, were analyzed according to CDC growth standards. Anthropometric parameters and clinical findings were systematically evaluated.

Results: Among the 100 patients analyzed (58% male, 42% female), the average age at clinical diagnosis was 6.46 years old. The majority (84%) presented with the common 3 Mb deletion. Key findings included short stature (26%, mean height z-score: −1.2), weight variability (28% underweight, 7% overweight), and microcephaly (19%). Cardiovascular anomalies were the predominant feature (76%), with ventricular septal defects (53%) and tetralogy of Fallot (30%) being most prevalent. Developmental challenges were common, including speech delay (69%), motor delay (57%), and intellectual disability (65%). Notably, 17% of cases lacked congenital anomalies at birth, with symptoms manifesting later in life.

Conclusion: This cohort represents the largest single-center dataset on 22q11.2 deletion syndrome in Brazil, highlighting phenotypic consistencies and unique regional patterns. These findings contribute to the global understanding of this syndrome, emphasizing the need for early diagnosis and tailored management strategies.

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P13.056.D Comprehensive reanalysis of septo-optic dysplasia supports more specific inclusion and exclusion criteria

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Background: Septo-optic dysplasia (SOD) was defined by combined absent septum pellucidum (ASP) and optic nerve hypoplasia (ONH) by De Morsier (1956). Later reports expanded the brain features to "midline prosencephalic" defects and eye anomalies to microphthalmia and colobomas. Pituitary insufficiency was added by Hoyt et al (1970), but diagnostic criteria comprising any 2 of 3 features were not stated until 2005.

Material and Methods: We studied a comprehensive review of the literature and a new cohort at our institution to re-construct features that clustered together.

Results: Early studies relied on limited understanding of brain development and limited brain imaging, assuming that isolated ASP shared pathogenesis with callosal defects and holoprosencephaly. We found that individuals with midline brain anomalies other than ASP or eye anomalies other than ONH were always atypical. And subjects with putative SOD and sequence variants in genes such as *HESX1* were also atypical. By focusing on typical features observed in most affected individuals, we propose new inclusion criteria consisting of any 2 of the following 4 abnormalities: (1) ASP, (2) ONH, (3) pituitary hypoplasia, and (4) malformations of cortical development (polymicrogyria, heterotopia). And importantly, we add new exclusion criteria comprising midline brain defects other than ASP (i.e. callosal defects), eye anomalies other than ONH, and pituitary insufficiency with normal pituitary structure.

Conclusion: Our new criteria will select a more homogenous group of individuals with low to nil yield from genetic testing, and better developmental outcome as other major brain malformations and genetic disorders will be excluded.

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I. Bacchi: None. **B. Miller:** None. **C.J. Billington, Jr:** None. **W.B. Dobyns:** None.

P13.057.A Microcephalies with recognizable phenotypes, although wide variable expressivity - a microtubule problem (case-series)

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Background: Here we present four unrelated families with *KIF11*-variants and one family with a *TUBB*-variant to illustrate the variable expressivity of kinesinopathies and tubulinopathies.

Patients and Methods: Four index patients were children with progressive microcephaly and variably present chorioretinopathy and lymphoedema. In one of the children, the diagnosis of *KIF11*-related microcephaly was clinically suspected based on the presence of chorioretinal atrophy and lymphedema. The fifth index patient was a woman referred to our clinic after she experienced two pregnancies with fetal anomalies and intrauterine fetal death. She was clinically diagnosed with Noonan syndrome in childhood based on the association of short stature with heart defect. The diagnosis was dismissed at the clinical genetic evaluation and Circumferential skin creases Kunze type (OMIM#156610) was strongly suspected based on her phenotype. WGS or WES Trio was performed in four families and a targeted analysis of a microcephaly-related gene panel was done in the patient with suspected *KIF11*-related microcephaly.

Results: In two out of four families, the *KIF11*-variants were inherited from parents with small head circumference without significant retinal changes, while de novo occurence was shown in the remaining two families. In the fifth family, the clinically suspected diagnosis was molecularly confirmed by the detection of a novel variant in *TUBB* by WES Trio in both the woman and the affected fetuses.

Conclusion: This case-series illustrates the importance of a specific phenotype-genotype association for the clinical interpretation of novel missense variants as well as the wide intrafamilial phenotypic variability of microtubule disorders.

C.O.I. Disclosure:

A. Ţopa: None. **J. Ottosson:** None. **C. Törnhage:** None.

P13.058.B A case of a de novo *GRIK2* pathogenic variant mimicking a mild Cornelia de Lange phenotype

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Background: Kainate receptors (KARs) are glutamatergic channels that are involved in multiple functions in the central nervous system. Mono-allelic pathogenic variants in *GRIK2* recently emerged as a cause of nonsyndromic neurodevelopmental disorder.

Material and Methods: Clinical assessment of a patient with a de novo *GRIK2* variant presenting with a clinical phenotype mimicking mild Cornelia de Lange spectrum.

Results: A 6-year-old boy presented with global developmental delay and dysmorphic features, consistent with a clinical diagnosis of Cornelia de Lange spectrum according to the scoring system proposed in the 2018 Consensus Statement. NGS-based gene panel for coesinopathies, performed both on blood and buccal swab, identified a variant of unknown significance (VUS) of maternal origin in *NIPBL*. Whole Exome Sequencing identified an heterozygous de novo variant in *GRIK2* [c.1969G>A, p(.Ala657Thr)], previously reported in patients with neurodevelopmental disorder and proven to alter the receptor kinetics via in vitro assays.

Conclusion: To our knowledge this represents the first case of a *GRIK2* pathogenic variant associated with a "syndromic" presentation. Whereas the neuronal expression of GRIK2 has been linked to brain development, little is known about the extra-cerebral functions of this receptor. No crosstalk between GRIK2 and coesinopathies molecular pathway has been established. Conversely, once considered a specific condition, recent clinical and genetic assessment have described a Cornelia de Lange spectrum, with classical and non-classical forms, and conditions with a phenotypic overlapping caused by variants in non-cohesin genes. Further investigation is needed to verify a causal link between *GRIK2* variants and the dysmorphological aspects herein described.

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P13.060.D Identification of a de novo Pathogenic Variant in *SET* in an Individual with Intellectual Disability and Dysmorphic Features

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Background: The *SET* gene encodes a nuclear protein involved in chromatin remodeling and histone acetylation regulation. It has recently been identified as the cause of Intellectual Developmental Disorder, Autosomal Dominant 58 (#MIM: 618106) (Stevens et al., 2018). Previously reported cases demonstrated mild dysmorphic features, including facial asymmetry, high palate, and prominent ears, though these features are not considered syndrome-specific. We present a 7-year-old boy whose phenotype aligns closely with prior descriptions.

Material and Methods: The proband, born at 40 weeks and 1 day (3622g), was first evaluated at 2 years and 5 months due to developmental delays, including head control at 5 months, walking at 24 months, and first words at 22 months. Growth parameters and head circumference were within normal ranges. Brain MRI showed no abnormalities. Chromosomal G-banding and microarray analyses were normal. Trio-based whole exome sequencing (WES) was performed using the Twist Comprehensive Exome, Mitochondrial DNA Panel Spike In and NovaSeq6000 platform.

Results: A de novo frameshift pathogenic variant in *SET* (NM_003011.4: c.128_131del: p.(Arg44Leufs*10)) was identified. This variant, registered as pathogenic in ClinVar and classified as pathogenic by ACMG guidelines, shares phenotypic features with previous reports, including mild ID, a prominent forehead, deep-set eyes, thin upper lip, a wide mouth, and noticeable dental gaps. Additionally, a cheerful and sociable personality was observed.

Conclusion: The phenotype overlaps with prior cases, suggesting *SET* variants may constitute a recognizable syndrome. Further accumulation of cases will expand the phenotypic spectrum and refine diagnostic frameworks.

C.O.I. Disclosure:

E. Nishi: None. **K. Yanagi:** None. **T. Kaname:** None. **N. Okamoto:** None.

P13.061.A A new case of a non-mosaic *PORCN* mutation in two male siblings with severe congenital anomalies overlapping Goltz-Gorlin syndrome

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Background: Variants in the *PORCN* gene cause the X-linked dominant condition Goltz-Gorlin syndrome or focal dermal hypoplasia (FDH). Features of FDH include striated pigmentation of the skin and ocular and skeletal malformations. This condition mainly affects females, as in non-mosaic males it usually causes lethality during fetal development. Most male cases reported to date have mosaicism, which is due to de novo mutations occurring after fertilization in the *PORCN* gene. Two cases of surviving male siblings with an inherited mutation in this gene have been described.

Material and Methods: Whole exome sequencing (WES) was performed in two male siblings with malformations including skeletal, ocular, renal defects and pulmonary hypertension.

Results: We report a novel non-mosaic variant, *PORCN*: c.1315T>G; p.(Trp439Gly), in two male siblings with malformations overlapping with Goltz-Gorlin syndrome. The phenotype of one of the brothers matches with one of the already described cases of a male with a non-mosaic variant, *PORCN*: c.749C>T; p.(Ser250Phe), presenting bilateral pyelocaliceal and ureteral hydronephrosis, bilateral vesico-ureteral reflux grade IV-V, coloboma, congenital pseudoarthrosis of the right clavicle, umbilical hernia and pulmonary hypertension.

Conclusion: While the pathogenicity of this variant remains to be further investigated, the presence of non-mosaic *PORCN* variants in males suggests that these are functionally mild mutations leading to an X-linked recessive mode of inheritance.

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P13.062.B Detection of a deep intronic variation in *ZEB2* by genomics studies thanks to the clinical expertise of the biologist, a case report of a familial recurrence

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Background: A poly-malformation syndrome including agenesis of the corpus callosum, hypospadias, and bilateral ureterohydronephrosis in a 26-week-old fetus, leads to medical termination. The feto-pathological analysis revealed retinal dysplasia and polysplenia. The second child, whose pre-natal ultrasounds were normal, presented with a severe neurodevelopmental delay, microcephaly, growth retardation, facial dysmorphia, a short corpus callosum on MRI, and asplenia.

Material and Methods: Full Genomic study in a quartet with the mother, father, terminated fetus, and sister followed by RNA sequencing.

Results: The facial dysmorphia of the fetus and its sister led the geneticist to suspect Mowat-Wilson syndrome. This allowed the identification of a deep intronic variation whose predictions favored a modification of the canonical splicing of the transcript. The fetus and its sister carry this variation (NM_014795.4 (ZEB2):c.808-572T>G) in the heterozygous state, and it is inherited from their mother in whom the variation was found at a frequency of 9%. She presented with no sign of Mowat-Wilson syndrome. RNA sequencing confirmed an alternative splicing, leading to an additional exon including a STOP codon, with truncating consequences.

Conclusion: The genome can detect intronic variations not covered by sequencing of gene panels or the complete exome, which are more difficult to identify and classify. The clinician as well as the biologist's expertise are essential for their interpretation. Four cases of recurrence of Mowat-Wilson syndrome were previously reported, and one case reported somatic mosaicism in a father. Clinical studies are currently underway in France to specify the fetal phenotype of Mowat-Wilson syndrome.

C.O.I. Disclosure:

J. Piotrowski: None. **E. Schaefer:** None. **A. Gouronc:** None.

P13.063.C Exome sequencing in the diagnosis of a complex pediatric case: from unclear prenatal findings to a definitive postnatal genetic diagnosis

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Introduction: We present a complex pediatric case involving a 7-month-old female with congenital ventriculomegaly, microcephaly, facial dysmorphism, and cerebral palsy characterized by frequent dystonic episodes.

Materials and Methods: Prenatal testing using Chromoseq was conducted due to ultrasound findings of ventriculomegaly. This test identified four copy number variations (CNVs) classified as variants of uncertain significance (VUSs). However, no further genetic testing was pursued due to the absence of counseling with a medical geneticist. Postnatally, whole exome sequencing (WES) was performed to investigate the underlying genetic etiology.

Results: WES identified a heterozygous de novo missense variant in the DDX3X gene: c.976C>T (p.Arg326Cys). This variant is absent from the gnomAD population database and is classified as pathogenic. It affects a highly conserved residue within the helicase domain of the DDX3X protein, which is crucial for RNA metabolism and neurogenesis during fetal cortical development. The identified mutation correlates with DDX3X-related neurodevelopmental disorder, a condition associated with intellectual disability, speech and language impairments, and motor abnormalities.

Conclusion: This case underscores the diagnostic utility of exome sequencing in complex pediatric cases where prenatal genetic tests fail to provide a definitive diagnosis. The identification of a pathogenic DDX3X variant facilitated a precise diagnosis, guiding clinical management and genetic counseling. Our findings emphasize the need for adequate genetic counseling and advanced genomic testing during the prenatal period to reduce missed opportunities for early intervention and personalized care.

C.O.I. Disclosure:

M. Panova: None. **H. Ivanov:** None. **I. Sotkova-Ivanova:** None.

P13.064.D Clinical and molecular findings in a novel 4q duplication syndrome, a case report

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Background: 4q duplication syndrome is a rare chromosomal abnormality characterized by the variable duplication sizes in long arm of chromosome 4. Syndrome manifests with a broad spectrum of phenotypic features, depending on the size and specific location of the duplication. Here, we present a case from Turkiye with a distinctive phenotype with a novel ~ 42.318 kbp duplication in 4q22.3q28.3 (95517323-137835086).

Material-Methods : Detailed family history and clinical examination were evaluated. The diagnosis was achieved through SNP-Array analysis.

Results: A 8-year-old girl was referred to our medical genetics outpatient clinic by dermatology due to skin findings and dysmorphic facial features. She was the second-born child from healthy non-consanguineous parents. She had postnatal growth retardation, global developmental delay, mild intellectual disability, hypotonia, constipation, attention deficit hyperactivity disorder, delayed speech and lisping and a developmental dysplasia of the hip. On physical examination, she had facial dysmorphism, microcephaly, localized hypopigmented hair strands, hypopigmented macules and xerosis on arms, hypertrichosis especially on extremities, left thumb hypoplasia and sacral dimple. SNP-Array revealed a 4q22.3q28.3(95517323-137835086) duplication with 16068 marker.

Conclusion: In the literature, 4q duplication syndrome has been reported with varying duplication sizes and associated with a broad spectrum of phenotypes. Through our novel case, we aim to contribute to the understanding of genotype-phenotype correlations and highlight the significance of phenotypic and genotypic variability in 4q duplication syndrome. This underscores the critical role of early intervention, precise genetic counseling, personalized management strategies, and long-term follow-up in optimizing patient outcomes.

C.O.I. Disclosure:

E. Bulut: None. **Z. Esener:** None. **&. Can:** None.

P13.065.A Complex phenotypic syndromic obesity: Co-occurrence of Roifman Syndrome and Weyers Acrofacial Dysostosis

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Background: Syndromic obesity is a rare condition involving obesity, short stature, dysmorphic features, neurodevelopmental delay, and systemic anomalies. Clinical evaluation and family history may be insufficient for diagnosis. We present a child with syndromic obesity carrying two distinct rare disorders, highlighting their combined effects on phenotype.

Material and Methods: Syndromic obesity is a rare condition involving obesity, short stature, dysmorphic features, neurodevelopmental delay, and systemic anomalies. Clinical evaluation and family history may be insufficient for diagnosis. We present a child with syndromic obesity carrying two distinct rare disorders, highlighting their combined effects on phenotype.

Results: Born to consanguineous parents at term with low birth weight (1850 g, <3rd percentile), she had recurrent infections requiring IV immunoglobulin. Walking was achieved at age 5, and speech was limited to two-word sentences. Examination revealed short stature (−5.26 SDS), obesity (BMI 20.2, 95th percentile), coarse facial features, synophrys, midface hypoplasia, conical canines, multiple frenula, brachydactyly, joint laxity, small extremities, simian creases, and partial toe syndactyly. WES identified a heterozygous *EVC* gene variant (c.1750del, p.Gln584ArgfsTer4), associated with Weyers acrofacial dysostosis, and a homozygous *RNU4ATAC* gene variant (n.16G>A), linked to Roifman syndrome. Retrospective phenotyping revealed that Weyers syndrome contributed skeletal and dental anomalies, while Roifman syndrome accounted for growth restriction, immunodeficiency, neurodevelopmental delay, and ocular findings. Their interplay created a diagnostically complex phenotype.

Conclusion: This case underscores the importance of detailed phenotyping and genetic analysis in syndromic obesity, particularly in consanguineous populations where multiple rare genetic disorders may co-segregate.

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P13.066.B Developmental defect of the urogenital tract, genua recurvata, hypotonic syndrome; using WES familial *CAV3* associated hyperCKemia was diagnosed

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Background/Objectives: The girl was examined after birth for a congenital developmental defect of the urogenital system: renal agenesis, cystic malformation in retroperitoneum, uterus bicornis, genua recurvata and an orthopedic defect of the limbs. SNP microarray, karyotype, NGS for rare disease and WES were done. The cause of congenital defects was not found. Subsequently, with an interval of 1 year, she comes for a hypotonic syndrome and an increased level of CK, hepatal enzymes in the serum, the neurologist sends to rule out a rare neuromuscular disease. After supplementing the anamnesis and clinical examination of the child and her mother (calf hypertrophy, negative EMG, hyperCK in both mother and child), the geneticist indicated a reanalysis of the whole-exome sequencing data.

Material and Methods: The patient was ascertained from a series of patients with paediatric-onset rare diseases with unknown genetic bases who underwent whole-exome sequencing at the Research Unit for Rare Diseases. Genomic DNA extracted from leukocytes of patient, her parents and healthy sibling was used for whole-exome sequencing on Novaseq 6000 platform (Illumina).

Results: Significant deleterious variant c.344A>G p.(Gln115Arg) in *CAV3* gene was detected; both mother and daughter carry it heterozygously, the DNA findings correspond to the phenotypic unit "Creatine phosphokinase elevated serum levels" OMIM (#123320).

Conclusion: A detailed evaluation of the patient´s phenotype and family history led to the discovery of the disease in the family. Authors highlight important reanalysis of WES data in a newly emerging clinical problem, segregation of the allele with the disease in the family.

C.O.I. Disclosure:

D. Grecmalova: None. **A. Hladikova:** None. **L. Noskova:** None. **M. Zikanova:** None. **S. Kmoch:** None.

P13.067.C Identification of Homozygous Variants in *PTK7* and *HSPG2* in a Neonate with Neural Tube Defect and Skeletal Anomalies

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Background: Neural tube defects (NTDs) are among the most common congenital malformations, resulting from the failure of neural tube closure between days 18–26 of embryonic development. The etiology involves both genetic and non-genetic factors, with folate metabolism and planar cell polarity signaling being key genetic pathways.

Material and Methods: Despite periconceptional folate supplementation, a newborn with meningomyelocele, born to consanguineous parents, was referred to our clinic. The infant presented with additional anomalies, including respiratory distress, costovertebral anomalies, intestinal malrotation, and cerebellar hypoplasia. The family history included a previous fetus with NTD and abdominal wall defect. Chromosomal microarray (CMA) and whole exome sequencing (WES) were performed from the patient's blood samples.

Results: CMA was normal. WES identified rare homozygous missense variants in the *HSPG2* (NM_005529.7: c.11648C>T, p.Ser3883-Leu) and *PTK7* (NM_002821.5: c.2648T>C, p.Leu883Pro) genes.

Conclusion: The clinical significance of the variants in *HSPG2* and *PTK7* remains uncertain. Although the patient's phenotype shares some similarities with *HSPG2*-associated conditions, such as neural tube defects (NTDs) and skeletal anomalies, it does not align with the typical *HSPG2* related phenotypes. The *PTK7* variant, involved in planar cell polarity and the Wnt signaling pathway, has not been previously reported in the homozygous state. Mouse studies suggest that biallelic *PTK7* loss-of-function variants lead to phenotypes resembling those observed in this patient and the fetus, including NTDs, abdominal wall defects, and costovertebral anomalies. Ongoing efforts to isolate DNA from autopsy tissues of the affected fetus for Sanger analysis may elucidate the role of observed phenotype.

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P13.068.D Clinical heterogeneity of the *GBE1* gene associated diseases

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Background: Biallelic pathogenic variants in the *GBE1* gene are implicated in two distinct clinical conditions: Glycogen Storage Disease Type IV (GSD IV; OMIM: 232500) and Adult Polyglucosan Body Disease (APBD; OMIM: 263570). GSD IV is characterized by a range of symptoms, involving the hepatobiliary or neuromuscular systems, with early childhood occurrence. APBD is primarily associated with the upper and lower motor neurons lesions, with clinical manifestations emerging after the age of 40.

Material and Methods: Clinical data analysis, Whole Exome Sequencing (WES) and Sanger sequencing for variants validation.

Results: An 11-year-old boy was referred for genetic counseling due to gait disturbance symptoms present since the age of 7. The patient had cirrhosis and underwent liver transplantation at age 4. Transaminase levels remained elevated even after transplantation, and electromyography was normal. Examination revealed signs of spastic paraplegia. Genetic analysis identified a previously reported pathogenic variant in the GBE1 gene (NM_000158.4: c.1543C>T p.(Arg515Cys)) and an unreported variant (NM_000158.4: c.1240G>A p.(Val414Ile)) in a compound-heterozygous state. The second patient, a 15-year-old boy, presented with similar complaints. Retrospective data revealed delayed motor development from birth, cirrhosis since age 3 followed by liver transplantation, and spastic paraplegia since age 9. The proband also had elevated transaminase levels after transplantation, along with cholestasis and low glucose levels. Electromyography showed a muscular type of peripheral neuromotor damage. WES identified the same variant as in the first patient (GBE1: c.1240G>A p.(Val414Ile)) in a homozygous state.

Conclusion: These cases demonstrate a novel combined presentation of two *GBE1*-associated phenotypes in children.

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P13.069.A Two novel cases with *PIGQ*-related GPI-anchor deficiency: expanding of the genotype and phenotype spectrum

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Background: The glycosylphosphatidylinositol (GPI)-anchor is a glycolipid that anchors proteins to the eucaryotic cell surface. An anchoring process is a posttranslational modification of at least

150 molecules with various functions. Biallelic causal variants in the PIGQ gene (OMIM: *605754) are associated with the type of GPI-anchor deficiency called Multiple congenital anomalies-hypotonia-seizures syndrome 4 (MCAHS4, OMIM: #618548).

Material and Methods: We present two novel cases of MCAHS4 with two novel variants in the *PIGQ* gene, detailed phenotyping, and a review of all published cases so far. For deep gestalt analysis, we used GestaltMatcher and investigated its potential use in diagnosing MCAHS4 patients.

Results: We found two novel variants in the *PIGQ* gene: frameshift c.1092dupC, p.(Phe365LeufsTer78) and VUS missense c.1370T>G, p.(Leu457Arg), which pathogenicity was proved by a functional study on Chinese hamster ovarian cells. After reviewing all 13 already diagnosed MCAHS4 patients, we found that attacks of rhabdomyolysis induced by a febrile infection were documented only in our patient. Facial dysmorphia (coarse features, anteverted nares, and open mouth) seen in all analyzed MCAHS4 patients seems specific. Moreover, GestaltMatcher proved that MCAHS4 patients shared a similar facial phenotype.

Conclusion: In the presented work, we expand the genotype spectrum by describing two novel causal *PIGQ* variants. In MCAHS4 patients, it is appropriate to consider attacks of rhabdomyolysis because of their life-threatening complications. GestaltMatcher seems to be an effective diagnostic tool in MCAHS4.

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P13.070.B An atypical form of Peters Plus syndrome without Peters anomaly: Expanding the *B3GLCT*-related phenotype with skeletal involvement

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Background: Peters Plus syndrome (PTRPLS) is an autosomal recessive disorder caused by *B3GLCT* variants [MIM* 610308], characterised by Peters anomaly, neurodevelopmental impairment, short stature, limb anomalies and/or cleft palate. Extraocular manifestations, especially skeletal defects, remain largely understudied.

Material and Methods: We present the case of an individual with a homozygous pathogenic variation in *B3GLCT*, exhibiting syndromic features, including skeletal involvement, neurodevelopmental disorder and atypical ocular manifestations in the absence of Peters anomaly.

Results: A 37-year-old case of unrelated parents presented with severe intrauterine growth restriction, ocular anomalies (bilateral iris chamber atrophy, severe myopia, glaucoma, retinovitreal degeneration, unilateral macular coloboma, nystagmus), skeletal involvement (short and broad hands and feet with brachydactly and brachymecarpia/tarsia, early-onset severe osteoarthritis, and narrow cervical canal) with short stature (137 cm), cerebral malformations (partial agenesis of corpus callosum, triventricular hydrocephalus), intellectual disability, autism spectrum disorder, and hearing loss. Trio exome sequencing identified the recurrent pathogenic variant in *B3GLCT*, NM_194318.4:c.660+1G>A at homozygous state.

Conclusion: While *B3GLCT* variants are linked to PTRPLS, extraocular features, particularly skeletal involvement, remain underreported. This case highlights an expanded phenotype with significant skeletal abnormalities, reinforcing the need to refine PTRPLS clinical delineation. We call for collaborative efforts to identify additional cases and better characterize skeletal manifestations associated with *B3GLCT* variants.

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P13.071.C Frontonasal dysplasia with creased nasal ridge (FNDCN): a member of the Recurrent Constellation of Embryonic Malformations (RCEM) group? Report of four new patients

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Background: we reported frontonasal dysplasia with creased nasal ridges (FNDCN) as a distinct entity among frontonasal dysplasia in 2017. This publication described the phenotype of eleven patients, including three discordant monozygous or dizygous twins. The etiology is unknown so far. In 2020, the concept of "recurrent constellation of embryonic malformations (RCEM)" has been suggested for a group of recurrent malformations without genetic basis, including the more frequent VACTERL and oculo-auriculo-vertebral syndrome, characterized among others by an excess of discordant monozygotic twins. RCEM are believed to share a common pathogenesis, as supported by the recent identification of a common DNA methylation episignature.

Material and Methods: following our publication describing FNDCN, we were contacted by colleagues from Maxillofacial or Genetics teams to report on four novel patients. Trio genome sequencing (GS) was performed in one patient.

Results: four unrelated individuals, aged 2 to 39 years, presented with the typical and recognizable facial aspect of FNDCN, including characteristic creased nasal dorsum, absent or bifid tip and hypertelorism. Abnormal olfactory bulbs and dehiscent basal skull were present in one individual each. Three patients were issued from twin pregnancies, all co-twins being unaffected. GS performed in one patient was non-contributive.

Conclusion: FNDCN is a rare entity, in which is observed an excess of twins (6/15 in total, 40%) and no familial recurrence. This report raises awareness on this condition and suggests its belonging to the recently conceptualized RCEM group. The identification of more patients will pave the way to the understanding of their common pathophysiology.

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P13.072.D Trio-Wes Analysis of Two Goldenhar Families and Two Candidate Genes

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Background: Goldenhar syndrome or Oculo-auriculo-vertebral spectrum (OAVS) is due to an abnormal development of first and second branchial arches during embryogenesis.OAVS is characterised by hemifacial microsomia associated with 3 main clinical findings auricular, ocular and vertebral malformations.In this study, we examined two OAVS patients with Trio-WES analysis.

Material and Methods: After DNA isolation from peripheral blood lymphocytes with the QIAampDNA mini kit, WES was done with the illumina NovaSeq Platform.Raw data was analyzed using the Sophia DDMR data analysis platform.

Results: Clinical findings of Patient 1 showed unilateral microtia and external auditory canal atresia, mandibular hypoplasia, facial asymmetry and macrostomia.Patient 2 had hydrocephalus in prenatal period.He had unilateral anophtalmia, unilateral microtia and external auditory canal atresia, mandibular hypoplasia, syringomyelia, unilateral hand polydactyly, AVSD, epilepsy, persistent pneumonia.In cranial MRI corpus callosum dysgenesis, polymicrogyria was detected.Both patients' chromosomal and aCGH analysis were normal.As a result of the trio-WES analysis, we have found *ARID3B* c.1523-1G>T and *FBLN1* p.Cys248Serfs*56 heterozygous novel de-novo variants in patient 1 and patient 2, respectively.

Conclusion: Recent studies have shown that FBLN1 and ARID3B are associated with FGF pathway and its functions include embryonic development and neural crest cell (NCC) maintenance, NCC migration as well as patterning of structures formed from NCC such as branchial arches and cranial nerves.ARID3B has been associated with OAVS in a genomewide association study.Knockout ARID3B animal modelling studies have shown that branchial arch, neural tube and otic vesicle development were disrupted.-Further studies are needed to uncover the relationship between these two genes and OAVS

C.O.I. Disclosure:

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P13.073.A Refining the ALKAS phenotype: first evidence of reproductive tract anomalies

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Background: Al Kaissi syndrome (ALKAS, #617694) is a rare developmental disorder characterized by developmental delay, growth retardation, facial dysmorphism and spinal malformations. Biallelic mutations in *CDK10*, encoding cyclin-dependent protein kinase-10 (#603464) causes this disorder. CDK10 forms a heterodimer with Cyclin M, a complex critical for cell proliferation and regulation of ciliogenesis. Here, we present the first adolescent female with ALKAS, expanding the clinical spectrum to include reproductive tract anomalies.

Methods and Results: The proband, 16-year-old female born to consanguineous parents, presented with microcephaly, intellectual disability, attention-deficit/hyperactivity disorder, articulation defects, wide-based gait and hearing loss. Facial dysmorphism was notable for telecanthus, hypertelorism, narrow palpebral fissures, posteriorly rotated ears, high and narrow palate, pointed chin and single palmar transverse creases. Skeletal abnormalities included torticollis, kyphoscoliosis, C2-C3 vertebral fusion and sphenoid fibrous dysplasia. Cranial magnetic resonance imaging revealed corpus callosum dysgenesis. Intriguingly, the proband exhibited menstrual irregularities and polymenorrhea, with an enlarged uterus relative for age and endometrial hyperplasia observed on suprapubic ultrasonography. Genetic workup demonstrated a 46,XX karyotype and no pathogenic copy number variation on microarray analysis. Whole exome sequencing identified a homozygous frameshift mutation in *CDK10* (c.520_521del, p.Lys174Glyfs*34), classified as disease-causing.

Conclusion: Recent studies indicate that CDK10/Cyclin-M deficiency disrupts ciliogenesis, leading to abnormal primary cilia morphology. This report, presenting the 12th known ALKAS family, suggests that reproductive tract anomalies may be a novel phenotypic feature of ALKAS, mirroring features of certain ciliopathies. Recognizing ALKAS as a potential ciliopathy could refine clinical management, uncover underlying molecular mechanisms and pave the way for targeted therapeutic strategies.

C.O.I. Disclosure:

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P13.074.B *PIK3CA* Related Overgrowth: a diverse case series from Romania

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Background: *PIK3CA*-Related Overgrowth Spectrum (PROS) refers to a heterogeneous group of overlapping syndromes characterized by segmental excessive growth and other clinical features such as brain abnormalities, vascular malformations, cutaneous manifestations and skeletal dysplasia.

Material and method: Herein we report 4 patients with PROS diagnosed using next generation sequencing gene panels from skin biopsy or buccal swab.

Result: Concise clinical description and variants identified were as follows: (1) a 4 year-old female patient with neurodevelopmental delay, obesity, hypothyroidism and postaxial polydactyly (*PIK3CA* NM_006218.4:c.2740G>A,p.Gly914Arg, Pathogenic), (2) a 13 year-old male with facial dysmorphic features, neurodevelopmental delay, macrocephaly, obesity and right lower limb hypertrophy (c.3061T>C,p.Tyr1021His, Pathogenic), (3) a 23 years old female with verrucous lymphovascular malformation of lower right limb (c.1625A>T,p.Glu542Val, Pathogenic)-treated with Sirolimus, and (4) a 4 years old male with right hemihypertrophy and mild meurodevelopmental delay (c.1412C>T,p.Pro471Leu, Likely Pathogenic). Clinically significant missense variants in *PIK3CA* gene were identified with variable variant allele frequency in studied tissues. Family history was negative for all.

Conclusion: PROS disease burden is significant, most patients presented neurodevelopmental delay aside from overgrowth. In the light of treatment options acting on the PI3K-AKT-mTOR pathway, somatic molecular diagnosis is crucial.

C.O.I. Disclosure:

A. Chirita-Emandi: None. **C. Munteanu:** None. **N. Andreescu:** None.

P13.075.C Phelan-McDermid 22q13.31 deletion originating from a maternal balanced translocation

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Background: Phelan-McDermid syndrome (PMS) is a rare genetic disorder characterized by developmental delay, hypotonia, absent/delayed speech, congenital heart defects and urogenital malformations. PMS is usually caused by telomeric deletions (from 160 Kb to 9 Mb) on the long arm of chromosome 22 either arising de novo or, because of inherited unbalanced translocations detected after parental conventional karyotyping.

Material and Methods: An 1-year-old female referred with hypotonia, absent speech, hydronephrosis, joint hypermobility and mitral valve prolapse underwent Whole Exome Sequencing (WES) and chromosome analysis. WES used xGen Exome Research v2 kit (IDT) for library preparation and NextSeq-500 system (Illumina) for sequencing. Bioinformatic analysis, with VarSome Clinical and ExomeDepth softwares, enables detection of both nucleotide and copy number (CNVs) variants, classified according to the ACMG and ClinGen recommendations. Further investigation included conventional karyotype on the patient and her parents.

Results: Heterozygous CNVs, comprising a 6.3 Mb pathogenic deletion (DEL:chr22:44892717-51220722, hg19) on chromosome 22q13.31-q13.33 and a 690.2 Kb duplication (DUP:24796957-25487163, hg19) of unknown significance (VUS) on 13q12.12, were detected by ExomeDepth. Subsequent chromosome analysis confirmed the deletion, and a diagnosis of PMS, in the patient, and indicated that the apparently unaffected mother carries a balanced translocation involving chromosomes 13 and 22 [46,XX,t(13;22)(q12.12;q13.3)].

Conclusion: Combined molecular and chromosomal analysis allowed case resolution and PMS diagnosis. Unbalanced segregation of reciprocal translocations generates CNVs in offsprings with rare genetic diseases and, despite the obvious benefits of WES, conventional karyotype can still contribute valuable information towards elucidation of escaping events and underlying mechanisms.

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P13.076.D Microcephaly Represented with Deletion of *SHH* and *DPP6* Genes on Unbalanced Segregation of Translocation t(7;11)(q34;q24.1)

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Background: Microcephaly affects one in every 30,000 to 250,000 births globally where the brain size and volume are less than the 3rd percentile. As a structural birth defect, it is commonly accompanied by a genetic component resulting in developmental delay and intellectual disability.

Material and Methods: Patient was born at 36 weeks; she was consulted for genetic examination at newborn intensive care unit. She had a head circumference of 32 cm (2nd percentile) with narrow forehead, frontal bossing, downslanting palpebral fissures, narrow nose root and tip, retrognathia, short neck, large ears and cutis marmaratus. She developed seizures and acute brain infarct at left parietooccipital lobe on MRI at one month. Karyotyping, whole exome analysis (WES) and microarray analysis were offered.

Results: Prenatal screening with non-invasive prenatal test (NIPT) revealed a microdeletion on chromosome 7 (7q34-q36.3) of 17,6 Mbp in size at 11th week of gestation. USG screening at 22 weeks was normal. The first microcephalic evaluation was reported at 32nd week on obstetric controls.Karyotyping results were 46,XX. WES analysis did not reveal any pathogenic variants except copy number analysis confirmed the NIPT results with additional duplication on chromosome 11 (11q24.1-q25). Microarray analysis result was arr[GRCh38] 7q34q36.3 (141,927,349_159,327,017)x1; arr[GRCh38] 11q24.1q25 (122,584,812_135,068,576)x3 which includes deletion of *SHH* and *DPP6* genes.

Conclusion: When there is a result of NIPT test with deletion/duplication, CNV or microarray analysis is mandatory for proper segregation analysis and correct prenatal/postnatal diagnosis of underlying translocation. Here we present a novel case of *DPP6 and SHH* haploinsufficiency with microcephaly.

C.O.I. Disclosure:

I. Sunar: A. Employment (full or part-time); Significant; Metagentech.

P13.077.A Renal Complications in a Patient with ITPR1-Related Spinocerebellar Ataxia

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Background: Spinocerebellar ataxia type 29 (SCA29) is a non-progressive cerebellar ataxia with autosomal dominant inheritance. It is characterized by childhood-onset hypotonia, delayed motor development, and cognitive impairment. Affected individuals exhibit cerebellar dysfunction, often with cerebellar atrophy on neuroimaging. A missense variant in *ITPR1* was determined to be responsible.

Patient: The patient is a 2-year-old girl. She is noted for cerebellar hypoplasia, hypoplasia of the right kidney, left renal hydronephrosis, psychomotor developmental delay, and characteristic facial features. The Family history is unknown. Chromosomal microarray analysis did not reveal any significant variants. Because of the presence of cerebellar hypoplasia, the patient was suspected to have spinocerebellar ataxia and was referred to our hospital for whole exome sequencing.

Results: We obtained blood samples from the patient and her parents and performed whole exome sequencing. We found that the patient had a previously reported de novo heterozygous pathogenic variant in the *ITPR1* gene. The variant is identified as follows: NM_002222(*ITPR1*): c.7604T>A p. (Ile2535Asn). The variant has been confirmed by Sanger sequencing. The patient had kidney hypoplasia and hydronephrosis, which were not typically associated with SCA29. Exome sequencing did not reveal any other variants. The *ITPR1* gene has been reported to be associated with renal abnormalities, and it is possible that renal malformations may have occurred due to its effect on the kidney during the embryonic period.

Conclusion: We concluded that a pathogenic variant of *ITPR1* may cause renal malformations associated with SCA29.

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T. Takamatsu: None. **H. Okuno:** None. **G. Yamanaka:** None. **N. Miyake:** None. **H. Numabe:** None.

P13.078.B Clinical and genetic variability in two Armenian patients with DiGeorge syndrome features

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Background: In this report we present two cases with DiGeorge clinical features[1], but different genetic results. The 1st patient in the 39th week of gestation, was born via cesarean section. At the age of 1,5 months, a ventricular septal defect with high pulmonary artery pressure was detected. At the time of the latest consultation: 3,5 months, weight 0%, height 0%, head circumference 0%. He has prominent ears, a round nose, retrognathia, and syndactyly on the right foot. The 2nd patient was born in the 39th week of gestation. During pregnancy limb shortening was detected via ultrasound. At the age of 13 days old, the weight was at 0.9%, height 1.2%, and head circumference 7.2%. The facial features include a narrow forehead, upslanting palpebral fissures, hypertelorism, flat nose with an upturned tip, long philtrum, thin lips, downturned corners of the mouth, short neck, and shortening of the limbs. The patient has tetralogy of Fallot.

Methods: For DiGeorge syndrome, the first patient underwent karyotyping[2] and FISH[3], whereas for the second patient an array CGH was performed.

Results: Studies of the 1st patient revealed 46,XY, t(17,22)(q21.3;q13.2) ish22q13.3(D22S75, TBX1, Tuple)x2. During FISH testing no deletions were detected in D22S75, TBX1, and Tuple genes. The results of the 2nd patient were arr[GRCh38] 8q24.22q24.3(132180770_145076860)x3, 20p13q11.23(68448_38132166)x3.

Conclusion: To our knowledge, there are no described cases of DiGeorge syndrome with such genetic changes. However, as the patients had characteristic phenotypical features, DiGeorge syndrome can't be excluded. Further investigation such as WES is needed to find the pathogenic variation.

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P13.079.C A rare case of de novo mosaicism of isochromosome 18q and 18p deletion

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Background: Cellular mosaicism involving an isochromosome 18q and a deletion 18p is an extremely rare chromosomal anomaly, with only nine reported cases, including four in a prenatal context and five in living children. Isochromosome 18q results from duplication of the long arm of chromosome 18, leading to trisomy 18q and monosomy 18p. Deletion 18p corresponds to partial or complete loss of the short arm of chromosome 18. In this work, we report the 10th case of i(18q)/del(18p) mosaicism diagnosed in a Moroccan girl and propose a hypothesis explaining its mechanism of formation.

Methods: A 4-year-old Moroccan girl, born to non-consanguineous parents, was referred to our medical genetics consultation for a dysmorphic syndrome associated with psychomotor developmental delay. A constitutional karyotype was performed in our laboratory using cultured lymphocytes obtained from patient's venous blood.

Results: The karyotype revealed i(18q)/del(18p) mosaicism, with 80% of cells carrying an isochromosome 18q and 20% of cells showing an 18p deletion. The parental karyotype, performed to investigate a possible balanced translocation, showed no abnormalities. Clinical analysis revealed phenotypic features similar to those described in isochromosome 18q syndrome, although with a milder expression of the typical trisomy 18q characteristics.

Conclusion: Mosaicism associating an 18q isochromosome and an 18p deletion represents an extremely rare chromosomal anomaly. The clinical phenotype is similar to that seen in i(18q) syndrome, although it expresses few of the typical features of 18q trisomy. Diagnosis relies on cytogenetic analysis, which is essential for guiding genetic counseling and adapting treatment.

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P13.080.D Valve insufficiency and aortic root dilatation: new signs in Non-FLHS SRCAP-related neurodevelopmental disorder

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We present the case of a 12-year-old boy who came to our attention due to intellectual disability and motor dyspraxia. At phisical examination he presented a slender physique with marfanoid habitus (height +2 SD, BMI 16.6), arachnodactyly, bilateral cavus feet, ogival palate, scoliosis, and left inguinal hernia. Cardiological evaluation with echocardiography disclosed mitral valve prolapse associated with mild insufficiency, bicuspid aortic valve with mild insufficiency and aortic root dilatation (z-score +3.7). Whole Exome Sequencing (WES) in trio identified a de novo heterozygous variant in exon 25 of *SRCAP* gene (c.5368_5371dupTTGA, p.Ser1791fs). The variant was not reported in dbSNP, ClinVar, GnomAD databases and was considered pathogenetics. *SCARP* truncating variants were originally associated with Floating-Harbor Syndrome (FLHS). More recently has been defined a new entity, called non-FLHS SRCAP-related neurodevelopmental disorder, linked to truncating variants outside FLHS region (ie exon 33 and 34) and characterized by developmental delay/intellectual disability, psychiatric symptoms, hypotonia and musculoskeletal abnormalities. The patients generally exhibit normal or tall stature and marfanoid habitus has been specifically reported in only one patient. To the best of our knowledge, cardiac abnormalities have never been reported in these patients but they haven't been precisely ruled out by echocardiography. Aortic root dilatation, bicuspid aortic valve and mitral valve could represent new features of non-FLHS SRCAP-related neurodevelopmental disorder, suggesting to carry out cardiological investigations in these patients.

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P13.081.A Expanding the phenotypic spectrum of 17q12 microdeletion syndrome: liver hemangioma as a novel feature

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Background:17q12 microdeletion syndrome is a rare disorder primarily associated with renal cystic disease, maturity-onset diabetes of the young type 5 and neurodevelopmental disorders. Beyond these common features, the syndrome manifests a wide phenotypic spectrum, including liver abnormalities, gastrointestinal features, and congenital heart diseases, which are rarely reported as components.

Material and Methods: Four patients were clinically evaluated to expand the phenotypic spectrum of 17q12 microdeletion syndrome. Three of the patients were children diagnosed with **renal cystic disease**. Along with this diagnosis, additional clinical features were identified: Case1 had **transposition of the great arteries**, which was surgically treated in infancy, along with **mild intellectual disability**; Case2 displayed **unusual dental development, liver hemangioma**, and **accessory spleen**; Case3 showed **macrocephaly** and **growth retardation**. Case4 was an adult patient diagnosed with **diabetes mellitus, sensorymotor neuropathy, liver hemangioma, elevated transaminase levels**, and **bipolar disorder**. Patients' genomic DNA was extracted from blood samples. The oligonucleotide microarray analysis was performed using Affymetrix Cytoscan Optima Array. Copy number variants (CNVs) were analyzed using ChAS4.2. CNV pathogenicity was interpreted based on ACMG guidelines.

Results: The array results of the patients, evaluated as patogenic, are presented in Table 1.

Conclusion: Due to the rarity of 17q12 microdeletion syndrome, it is important to evaluate patients systematically to prevent uncommon findings from being overlooked. In the literature, only one patient with 17q12 microdeletion syndrome has been reported to have liver hemangioma. Combined with our data, liver hemangioma may represent a newly identified phenotypic trait.

Table 1: Array results of the patients

CASE1	arr[GRCh38]17q12(36,466,620_38,254,553)x1
CASE2	arr[GRCh38]17q12(36,467,352_37,924,067)x1
CASE3	arr[GRCh38]17q12(36,134,106_38,214,937)x1
CASE4	arr[GRCh38]17q12(36,511,076_38,214,937)x1

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P13.083.C Thyroid analytical screening: a case-report emphasizing added-value in patients with intellectual disability

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Background: The *SECISBP2* gene encodes a key factor for incorporating selenocysteine into selenoproteins, essential for cellular redox regulation, thyroid hormone metabolism, and immune function. Biallelic deleterious variants in *SECISBP2* cause Abnormal Thyroid Hormone Metabolism 1 (THMA1, MIM #607693), a multisystemic condition with varying degrees of endocrine, metabolic, and neurological features. With fifteen patients reported to date, we describe a new case, aiming to expand knowledge regarding this ultra-rare condition.

Material and Methods: Case file consultation and literature review.

Results: A 21-year-old male, born to non-consanguineous parents with unremarkable family history. He presented microcephaly (3rd centile), dysmorphic features, hypotonia, moderate intellectual disability (ID), hearing loss, and proportionate short stature (1,61m). *FMR1* testing, array-CGH, and myopathy NGS gene panel were negative. Biochemistry blood-work showed persistently elevated CKs (ranging from 287 to 1473, for reference 24–204) and thyroxine (T4) (ranging from 14 to 17.3, for reference 5,91-13.2), with low triiodothyronine (T3) (ranging from 0.4 to 0.6, for reference 0,92-2.18). WES disclosed two novel truncating *SECISBP2* variants. Segregation studies confirmed the likely pathogenic variants were biallelic, establishing the diagnosis of THMA1 in our patient.

Conclusion: While the minor thyroid function abnormalities were acknowledged, their syndromic nature only became clear after WES analysis. This confirms the consensus benefit of high-throughput diagnostics in patients presenting complex multisystem features and in their re-appreciation. The combination of increased T4 with low T3 is an uncommon feature that provides an important etiology clue and shows added utility, beyond hypo/hyperthyroidism, of thorough thyroid assessment in ID patients.

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P13.084.D Novel Genitourinary Anomalies and Facial Dysmorphisms in a Patient with a Large Mosaic Duplication of Chromosome 11p

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Background: Duplications of chromosome 11p15 encompassing the BWS critical region have been associated with Beckwith-Wiedemann and Russel Silver Syndrome phenotypes, depending on parent-of-origin. Larger duplications may be associated with additional anomalies/comorbidities, but few duplications larger than 6 Mb have been reported in living individuals.

Results: We describe a 58-year-old female patient with a very large (>15 Mb) high-level mosaic duplication of 11p associated with an unbalanced derivative chromosome 21 (46,XX,der(21)t(11;21)(p15.2;p13)[24]/46,XX[6], arr[GRCh37] 11p15.5p15.2 (198510_15319228)x3) in peripheral blood. MS-MLPA to determine parent of origin is pending. Clinically, the patient clinically has striking facial dysmorphisms including short palpebral fissures/blepharophimosis, frontal upsweep, bulbous nasal tip, simple low set ears, and cranial nerve palsies (reminiscent of Nablus syndrome). She additionally has significant short stature (−5.7SD), rhizomelia and mild leg-length discrepancy, as well as striking genitourinary anomalies including underdeveloped,

hypoplastic external genitalia with a large, cavernous outlet. Imaging confirmed presence of a uterus. History includes natal teeth, epilepsy, and moderate intellectual disability, but she is otherwise well.

Conclusion: To our knowledge, this represents the oldest reported patient with a duplication of 11p of this magnitude. Moreover, this is the first report of substantial genitourinary malformations associated with 11p duplications in a female, and of facial features reminiscent of Nablus Mask-Like Facies Syndrome. Parent-of-origin is not yet known, but she does not clearly fit within either the BWS or RSS spectrums, and anomalies are much more significant. Mosaicism and/or position effect from adjacent chromatin at the translocation site may be contributing to viability and lack of life-limiting comorbidities.

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P14 Cytogenetics, Genome Variation and Architecture

P14.001.A Reclassification of splicing variants of uncertain significance

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Background: Variants in +/−1,2 canonical splice sites and other variants, such as splice region, missense, or synonymous variants can alter constitutive splicing. Such variants may produce (multiple) aberrant functional and/or non-functional transcripts. Furthermore, it can be time consuming for Clinical Scientists to revisit previously identified variants of uncertain significance (VUS).

Material and Methods: We perform an evaluation of spliceogenic variants in genes related to Gastrohepatology and Inherited Cancers in a cohort of NHS patients. We targeted VUS that are more likely to be reclassified following the Walker *et al*., 2023 ClinGen splicing SVI and the latest ACGS 2024 variant classification guidelines. Using a variety of in silico tools we compared the predicted splicing impact of variants within the same splice region. Finally, we fill a gap by creating new resources, such as a gene specific PVS1 decision tree, to aid splicing variant interpretation in NHS laboratories.

Results: Out of 7533 variants, we identified over 400 spliceogenic VUS. Applying PS1 classification criteria enabled us to reclassify 11 out of 24 VUS to likely pathogenic. We also show that different in silico tools can complement each other and we generated a *PKD1* PVS1 decision tree to ease variant interpretation.

Conclusion: Reclassification of 46% of the splicing variants will impact patient management, for example by allowing familial predictive testing. Production of resources on splicing variant classification will aid variant interpretation for clinical scientists. We also illustrate that the use of PS1 brings additional weight when assigning pathogenic criteria.

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P14.002.B A palindrome associated duplication-triplication of 16p13.3 causes cerebellar atrophy and progressive ataxia

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Background: Complex structural variants (SVs) are linked to regions containing DNA palindromes, motifs that can form hairpin structures predisposing to double-strand breaks.

Materials and Methods: We identified a novel, severe and recognisable neurodegenerative disorder associated with triplication of part of chromosome 16p13.3. Clinical and neuroradiological studies were undertaken to delineate the phenotypic spectrum. Analysis of data from microarray, short and long-read genome sequencing, constellation read technology and RNAseq was undertaken to fully characterise the molecular basis of the condition.

Results: Eleven affected individuals from nine families presented with progressive early-onset ataxia (10–23 years) and cerebellar atrophy. Additional variable features included intellectual disability, neurodevelopmental regression, peripheral neuropathy and scoliosis. In all families where genome sequencing and/or high-resolution array data were available (7/9), a triplicated segment was identified within the duplication, with central inversion in five cases. Parental segregation data, available for 7/8, showed the SVs to have occurred de novo or inheritance from a mosaic parent, with one additional multiplex family (three individuals). While nineteen duplications were identified in UK Biobank (500,000 individuals), no triplications were detected. The minimal overlap region included *ATP6V0C* and *AMDHD2*, both highly expressed in the cerebellum and RNAseq in blood showed a 4-fold increase in *ATP6V0C* expression. The proximal breakpoints of these SVs lie within a 144 kb palindromic repeat, which likely mediates the rearrangements.

Conclusion: We describe a novel neurodegenerative disorder caused by triplication of a region containing *ATP6V0C*/*AMDHD2*, likely mediated by a palindromic repeat. This is the second distinct neurodevelopmental disorder linked to this locus.

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P14.003.C Unravelling the diagnostic power of whole-exome sequencing for copy number variant detection

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Background: Whole-exome sequencing (WES) has emerged as a powerful tool for detecting single nucleotide variants (SNVs) and insertions/deletions (indels). Recent studies have shown that WES, paired with advanced computational methods, can also provide high-resolution copy number variant (CNV) detection, often surpassing traditional techniques in terms of accuracy and efficiency. Here we present four cases of CNV detection by WES in patients with various neurodevelopmental disorders and/or congenital anomalies, who remained undiagnosed after karyotyping and array-CGH (resolution 100 kb).

Material and Methods: WES was performed on Illumina NextSeq 2000 platform using Illumina DNA Prep with Exome Enrichment. Bioinformatic analysis, annotation and interpretation were performed on Franklin by Genoox platform using the human genome assembly hg19. Array-CGH results were retrospectively re-evaluated and quantitative Real-Time PCR (qRT-PCR) was used to validate WES findings.

Results: In 4/136 family trios, pathogenic or likely pathogenic CNVs, consistent with the patients' phenotype, were identified from WES: i) a ~6.15 kb copy number gain in *SLC12A3* (exons 9–14) found in trans with a pathogenic missense SNV, ii) a de novo heterozygous ~793 bp deletion in *POGZ* (exons 11–12), iii) a homozygous ~140 bp deletion in *IFT74* (exon2), and iv) a homozygous ~84 kb deletion in *PGM2L1* (exons 6–14). None of the CNVs were detectable by array-CGH, but all were successfully confirmed by qRT-PCR.

Conclusion: This study supports that detection of CNVs <100 kb yields an additional ~3% of resolved cases. Hence, CNV analysis incorporation into WES diagnostic pipelines is of outmost importance for comprehensive evaluation of patients and discovery of novel disease-associated variants.

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P14.004.D Spreading of X-chromosome inactivation into autosomal sequences in unbalanced X-Autosome translocations: predicting silencing and escape

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Background: Unbalanced X-autosome translocations are rare structural abnormalities that present unique challenges due to skewed X-chromosome inactivation and its spread into translocated autosomal regions. Assessing silencing in these regions aids phenotypic characterization and helps uncover genomic and epigenetic features that could predict silencing and escape.

Material and Methods: We studied three patients with unbalanced X-autosome translocations involving chromosomes 13, 15, and 21, confirmed by G-banding karyotype and chromosomal microarray. Using Long-read Sequencing and RNA-seq, we assessed haplotype-resolved DNA methylation and allele-specific gene expression profiles from peripheral blood samples. This allowed classification of autosomal genes as active/inactive. In silico, we annotated 43 genomic and epigenetic features in human embryonic stem cells for 101 active and 101 inactive translocated genes to develop a Random Forest Model (RFM) with RFM-based clustering that identifies and prioritizes underlying predictors of silencing and escape.

Results: Variable silencing was observed in 24–26% of autosomal genes across translocated regions, with allele-specific hypermethylation identified near promoters of some clinically relevant genes. The RFM (mean accuracy = 75%, p[accuracy>non-information rate] <0.05) revealed reduced genomic distance from XIST and association of polycomb features as predictors of silencing. Conversely, increased ALU density and gene density were associated with escape. Validation on an independent dataset of six unbalanced X-autosome translocations (N = 904 genes) yielded 80% predictive accuracy.

Conclusion: Our findings emphasize the interplay between genomic and epigenetic elements in predicting silencing and escape in unbalanced X-autosome translocations. Moreover, the RFM could be applied to predict inactivation status of clinically relevant genes in similar translocations.

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P14.006.B A novel 9q34.11 microduplication syndrome with neurodevelopmental disorder and recurrent dysmorphisms

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Background/Objectives: Deletions and pathogenic variants in *SET* and *SPTAN1*, included in 9q34.11 locus, are associated with neurodevelopmental disorders (NDDs) and intellectual disability. To date, duplications of the 9q34.11 region have not yet been reported as pathogenic. We report 11 individuals with overlapping 9q34.11 microduplications presenting with NDD and recurrent facial dysmorphic features.

Methods: Pediatric individuals with NDD were evaluated by Chromosomal Microarray Analysis. Cases were collected through DECIPHER and the ITHACA European Reference Network. Nine unaffected parents of children with NDDs were analyzed as controls.

Results: Eleven individuals aged 4 to 21 years with overlapping 9q34.11 microduplications exhibited NDD (11/11), intellectual disability (8/11), and autism spectrum disorder (4/11). The minimal region of overlap (chr9: 128679215-128770807 on GRCh38/hg38) encompasses *SET* and *ZER1*, with 10/11 cases also involving *SPTAN1*. Two individuals developed epilepsy; mild dilatation of the renal pelvis was noted (3/11). Dysmorphic features including midface hypoplasia and thin lips, were observed in most cases. Among unaffected controls, 8/9 carried a partial duplication of *ZER1* and 1 carried a whole duplication of *SET*.

Conclusion: We report 11 individuals with 9q34.11 microduplications encompassing *SET* and *ZER1* in all cases and *SPTAN1* in 10/11 cases. The only case without *SPTAN1* duplication also carried a pathogenic 16p11.2 deletion. Altogether, these results suggest that increased gene dosage of *SPTAN1* is responsible for a novel 9q34.11 microduplication syndrome presenting with NDD and recurrent dysmorphisms.

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P14.007.C Hi-C and RNA-seq analyses on hiPSC-derived astrocytes allow to further decipher pathomechanisms in Autosomal Dominant adult-onset demyelinating LeukoDystrophy (ADLD)

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Background: ADLD is a rare neurodegenerative disorder caused by LMNB1 overexpression due to gene duplications (classical ADLD) or noncoding deletions in the locus (atypical ADLD). Our group collected > 20 families worldwide carrying structural variants (SVs) in the *LMNB1* locus and reported strong clinical variability, even among patients carrying duplications of the entire *LMNB1* gene, ranging from classical and atypical ADLD to asymptomatic carriers. We performed Hi-C and RNA sequencing on ADLD fibroblasts, providing preliminary insights into ADLD pathomechanisms and the genotype/phenotype correlation of SVs at the *LMNB1* locus. Here, we aim to validate these findings in disease-relevant cellular models, namely hiPSC-derived astrocytes.

Material and Methods: We generated hiPSC-derived astrocytes from four subjects carrying a SV in the *LMNB1* locus (a classical ADLD and two atypical ADLD patients, and an asymptomatic carrier) and a healthy control. Hi-C and RNA seq were performed on 1 million astrocytes per sample following standard protocols.

Results: We validate that: classical ADLD is due to an intra-TAD duplication, resulting in a simple gene dose gain; atypical ADLD is caused by *LMNB1* forebrain-specific misexpression due to SVs encompassing a TAD boundary. Finally, in the asymptomatic subject we identified an inter-TAD duplication, which extends centromerically and crosses two boundaries.

Conclusion: These results emphasize the critical role of TAD structure in mediating the pathogenicity of SVs in the *LMNB1* locus and further show that a duplication encompassing the *LMNB1* gene is not sufficient per se to diagnose ADLD, with implications for ADLD diagnosis and genetic counseling.

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P14.008.D Comprehensive reconstruction of Ring14 chromosome alterations with Hi-C and Long Read Sequencing

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Background: Ring14 syndrome (R14S) is a rare neurodevelopmental disorder with a complex phenotype, including drug-resistant epilepsy. R14S is caused by the rearrangement of chromosome 14 into a ring-shaped structure formed by two breakpoints fused with terminal deletions. This genetic loss doesn't explain some clinical features, e.g., individuals with similar 14q linear deletions don't exhibit epilepsy[1]. Our project aims to characterize the molecular pathogenesis of R14S using genome-wide chromosome conformation capture (Hi-C) and define R14 breakpoints and ring-associated rearrangements with long-read sequencing (LRS), exploiting the complete T2T-CHM13 human genome assembly[2] where acrocentric chromosomes are resolved.

Methods: We analyzed cell lines from 10 R14S patients and 5 parents from the Telethon Network of Genetic Biobanks. We performed Hi-C on the entire cohort and whole-genome LRS with Oxford Nanopore Technology (ONT) on a subset of samples. Hi-C data were processed using HiCPro[3] and FitHiC2[4]. LRS reads were aligned with Minimap2[5] and variant calling was performed with Sniffles[6] and Spectre[7]. PEPPER-Margin-DeepVariant pipeline[8] was applied for haplotag.

Results: Using Hi-C, we reconstructed ring contacts between 14p and 14q ends and detected terminal 14q deletions (100 Kb-5 Mb) identified by a-CGH. LRS confirmed the intra-chromosomal junctions observed with Hi-C, showing circularization involved genetic rearrangement at both chromosomal ends.

Conclusion: Our study highlights the innovative use of Hi-C and LRS to characterize Ring14 chromosomes. These techniques, while well-established for genome assembly, have not been applied to human ring chromosomes. By combining Hi-C's structural resolution with LRS, we offer a novel approach for studying chromosomal alterations in R14S.

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P14.009.A Cohort of 90 patients mainly affected by neurodevelopmental delay analyzed using optical genome mapping (OMG) technique in constitutional cytogenomics

Martine Doco-Fenzy[1], Emma N. Benbakir[2], Wallid Deb[2], Bertrand Isidor[2], Olivier Pichon[3], Emilie Landais[1], Tony Yammine[1], Celine Poirsier[1], Faezeh Vasheghani[2], Mathilde Nizon[2], Nathalie Bednarek[4], Sandra Mercier[2], Marie Vincent[5], Sylvie Jaillard[6], Sylvie Odent[7], Sylvie Bernard[8], Nicolas Gruchy[9], Mylene Dexheimer[10], Celine Bonnet[10], Nathalie Ledu[11], Noemie Celton[11], Paul Gueguen[11], Vincent Jauffret[8], Clemence Jacquin[1], Jonathan Levy[12], Agnes Guichet[13], ***Stephane Bezieau***[2]

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Optical genome mapping (OGM) is an innovative technology based on automated fluorescence optical imaging of ultra-long DNA molecules. This whole-genome approach enables a rapid analysis of structural variants (SV) and their spatial organization. Ninety patient samples, including 85 peripheral blood and 5 amniotic fluids, were collected between 2022 and 2024 and analyzed using OGM in the cytogenetics department. Among these, 30 cases with SVs previously identified by routine diagnostic methods (karyotyping, CMA, genome sequencing) were reanalyzed using OGM. Additionally, 60 cases with undiagnosed neurodevelopmental delay were investigated to identify any potential pathogenic SV. Except for two families analyzed as duo/trio cases, the analyses were performed as solo studies. In our cohort, OGM demonstrated a 97.1% concordance with the SVs identified by prior diagnostic methods. Furthermore, OGM contributed to a better spatial understanding of these chromosomal aberrations in 65% of cases. Candidate SVs were discovered in 33% of cases including eight patients with at least one likely pathogenic or pathogenic SV. Overall, we analyzed a broad spectrum of chromosomal abnormalities such as aneuploidies, deletions, duplications, inversions and insertions, triplications, translocation-derived chromosomes, ring chromosomes and markers, inversion-duplication-deletion and complex rearrangements. We compared them with other techniques in order to validate the protocol using OGM. The results of other techniques will be reported in comparison. Furthermore, no pathogenic variants were identified in 29 cases. This study enhances our knowledge of OGM useful applications in constitutional rare diseases, highlighting its ability to perform high-resolution SV characterization as well as its advantages and limitations.

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P14.010.B Chromosome 17q12 deletion and duplication in the Irish population

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Background: The recurrent chromosomal region 17q12 is linked to diverse clinical manifestations, particularly neurodevelopmental issues and organ-specific abnormalities. Deletions of 17q12 are strongly associated with renal and genitourinary anomalies, as well as maturity-onset diabetes of the young type 5. In contrast, duplications exhibit variable phenotype ranging from normal to severe intellectual disabilities, developmental delays, and neuropsychiatric conditions. This study explores the clinical phenotypes associated with 17q12 deletions and duplications in an Irish cohort.

Material and Methods: 36 cases were identified through the cytogenetic and clinical database of the Department of Clinical Genetics, Children's Health Ireland, searching between 2013 and 2024: 20 with deletions and 16 with duplications. Array comparative genomic hybridization (Array-CGH) and next-

generation sequencing-based copy number variant (CNV) analysis were utilized. Platforms included 120 kb and 180 kb CGH arrays (Agilent) and Illumina NovaSeq6000. Ethical approval was obtained (REC-503-24).

Results: Findings showed that 75% of deletion cases had antenatal findings, predominantly renal anomalies (85%), compared to only one duplication case with antenatal findings. Developmental delays were more pronounced in duplications than deletions, with significant differences in gross motor, fine motor, and speech delays. Neurodiversity and intellectual disabilities were observed in both groups, while more severe autism appeared more frequently with deletions, influencing school placement. Parental testing revealed that 80% of duplications were inherited compared to only 6% of deletions and parental phenotype were explored respectively.

Conclusion: This study highlights the multisystem and neurodevelopmental impact of 17q12 deletions and duplications. It also emphasizes the importance of parental segregation studies in guiding clinical management.

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P14.011.C Exploring Chromosome Instability: the DEB test beyond the diagnosis of Fanconi Anemia

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Background: Fanconi anemia (FA) is a rare genetic disorder characterized by chromosome instability (CI), particularly following exposure to DNA crosslinking agents, such as 1,2:3,4-Diepoxybutane (DEB). The DEB test, sensitive and specific, remains the gold standard for FA diagnosis due to its non-overlapping separation between positive and negative CI values. However, a subset of patients referred to our Laboratory for this test exhibited intermediate CI levels, i.e., between FA and control values, suggesting potentially reduced DNA repair capacity.

Material and Methods: A retrospective study was performed using CI levels from a cohort of patients evaluated for suspicion/exclusion of FA between 1993 and 2025. The study included three groups: healthy controls (n = 244), FA patients (n = 83), and non-FA patients, with molecular exclusion of FA (n = 813). CI assays were conducted using different concentrations of DEB. Statistical analyses were employed to compare CI levels across the groups.

Results: Within the non-FA group, we identified a subset of patients exhibiting intermediate CI levels. These included individuals diagnosed with inherited bone marrow failure syndromes (IBMFS), mostly Shwachman-Diamond syndrome, as well as patients without a definitive diagnosis.

Conclusion: Our findings demonstrate a spectrum of CI levels in non-AF individuals, emphasizing the clinical relevance of precise CI analysis beyond FA diagnosis. Further investigation is crucial to understand the underlying mechanisms of CI and its clinical implications. Of particular clinical significance are the IBMFS, where pre-transplant conditioning regimens for HSCT may require careful tailoring to minimize the risk of toxicity due to their potentially compromised DNA repair capacity.

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P14.012.D Integration of Long-Read Genome Sequencing with Prenatal Testing to Resolve Structural and Epigenetic Consequences of an X;1 Chromosomal Translocation

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Background: Resolving complex chromosomal anomalies traditionally requires multiple techniques, such as karyotyping, microarray, FISH, short-read genome sequencing and, if needed, epigenetic assays for X-chromosome inactivation (XCI). Long-read genome sequencing (LR-GS) provides a comprehensive, single-platform alternative. We present a case where LR-GS alone provided a complete genomic and epigenomic assessment of an unbalanced X;1 translocation in a prenatal sample initially detected by non-invasive prenatal testing (NIPT).

Material and Methods: NIPT identified a chromosome 1q gain. Conventional follow-up methods suggested an unbalanced translocation between chromosomes X and 1. Instead of using multiple assays, we performed nanopore LR-GS and used sniffles2 and spectre to resolve the precise structure of the translocation, and in house scripts to evaluate methylation alterations on chromosome 1 and X.

Results: LR-GS precisely delineated the breakpoints of the unbalanced X;1 translocation, identifying both a terminal Xq loss and terminal 1q gain. Additionally, direct DNA methylation analysis demonstrated skewed XCI, showing inactivation exclusively of the derivative X;1 chromosome (carrying the 1q gain), providing functional insight without the need for separate XCI testing. As the 1q gain derivate chromosome is inactivated, the possible effect of this gain remains unknown. This integrated approach allowed for characterization of both complex structural and epigenetic variant evaluation in a single experiment.

Conclusion: This case illustrates the potential of LR-GS as a single-platform alternative to conventional cytogenetic and molecular techniques. By integrating structural variant detection with direct XCI assessment, LR-GS streamlines genomic analysis, with the potential to improve diagnostic efficiency in (prenatal) genetic testing.

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P14.013.A An Emirati pangenome incorporating a diploid telomere-to-telomere reference

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Background: Reference data on genomic variation is at the basis of genetics research. To date, global pangenomes do not include

genomes from the Middle Eastern Region. To address this shortcoming, we provide an Emirati genome reference.

Material and Methods: The core of the reference is a diploid assembly with a QV of 60 that includes ten telomere-to-telomere chromosomes. This assembly is incorporated into a pangenome graph constructed of 52 additional high-quality, trio-based assemblies, half of which are trio-based. Advancements in sequencing technologies enabled chromosome-level accuracy, and all assemblies were evaluated for coverage, consistency, and structural integrity to ensure robust representation of challenging genomic regions.

Results: The Emirati pangenome reveals a similar level of genomic variation compared to the Human Pangenome Reference Consortium, thus filling a critical gap by including a Middle Eastern genome component. Its telomere-to-telomere chromosomes permit reliable detection of complex genomic regions, enhancing the identification of both global and population-specific variants. This resource offers improved insights into regional genetic diversity, an area often underrepresented in existing references. The successful assembly pipeline highlights the feasibility of producing similarly comprehensive pangenomes for other underserved populations.

Conclusion: Our Emirati pangenome provides an essential reference for global genomics research while emphasizing the importance of diverse ancestries. This high-quality, diploid assembly supports personalized medicine initiatives in the United Arab Emirates and beyond, paving the way for broader, more inclusive genomic discovery and application.

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P14.014.B De novo assembly of the H9 cell line

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Background: Human embryonic stem cell (hESC) lines such as the H9 cell line are widely used in research due to their differentiation potential making them an optimal platform for genome editing and developmental studies. The H9 cell line has been genome sequenced using short read sequencing but a full genome assembly is not yet available, hampering editing and precluding the study of repetitive regions.

Material and Methods: To generate a telomere to telomere (T2T) H9 assembly, we performed both PacBio and Oxford Nanopore Technology (ONT) long read sequencing. Assemblies have been generated using hifiasm and flye for PacBio and ONT, respectively. Verkko has been used to assemble PacBio reads with ultra long ONT data. The Nanopore-only T2T protocol consisting of two ultra long runs, one pore-C run and one assembly polishing run has also been tested.

Results: A regular ONT run leads to the assembly of 91% of the genome in 1,280 haploid contigs while a single PacBio run covers 93% of the genome with 322 and 318 haploid contigs. The use of ONT read correction and pore-C as well as nanopore only T2T are promising to further improve the de novo assembly and decrease the number of haploid contigs. We will present and compare those assembly results.

Conclusion: Long read sequencing allowed the full assembly of the H9 cell line, including the assembly of repetitive regions.

C.O.I. Disclosure:

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P14.015.C Alternative splicing site events in Arrhythmogenic Cardiomyopathy-related genes using in silico predictions and minigene assays

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Background: Splicing site variant (SSV) data and associated computational tools often exhibit limited sensitivity and specificity, offering only probabilistic estimates and imprecise evidence weighting for splicing findings. Arrhythmogenic cardiomyopathy (ACM) [OMIM#107970, ORPHA247], a rare inherited cardiac disorder, is predominantly caused by pathogenic variants in desmosomal genes, with 10–15% affecting noncanonical splice sites, auxiliary splice sites, and deep-intronic regions. This study sought to generate and apply minigene splicing assay for a prioritized set of splicing variants to refine their classification in accordance with ClinGen recommendations, ultimately improving diagnostic accuracy.

Material-and-Methods: Selected variants from 112 consecutive ACM patients were cloned into the pSPL3 plasmid to create a minigene, which was transfected into HEK-293T cells. Aberrant RNA transcripts were analyzed via reverse transcription and direct sequencing. When available, frozen tissue was used to confirm results.

Results: Among 15 rare SSVs identified in ACM-related genes, including *desmoplakin, plakophilin-2, desmoglein-2, desmocollin-2*, and *filamin-C*, 4 were located in canonical splice sites, while 11 were in non-canonical regions. Minigene assays detected alternative splicing events in 9 of these variants. Canonical variants predominantly resulted in exon skipping, whereas non-canonical variants led to alternative intron retention or abnormal exon deletion, revealing previously unrecognized molecular mechanisms. RNA analysis on frozen tissue confirmed the concordance between in vitro assay and natural splicing processes.

Conclusion: Approximately one-third of rare SSVs were reclassified based on in vitro loss-of-function evidence, enabling appropriate PVS1-assignment. Integrating functional validation with bioinformatics significantly improved pathogenicity assessment of splicing variants, enhancing diagnostic accuracy and clinical management of ACM patients.

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P14.017.A Breakpoint mapping in balanced chromosomal rearrangements allows insights into the molecular underpinnings of rare diseases

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Background: Balanced chromosomal rearrangements can often lead to the dysfunction of multiple genes located at or near the breakpoints. Among individuals with balanced rearrangements, approximately 6% exhibit phenotypic abnormalities attributed to various pathogenic mechanisms, including copy number variations (CNVs) undetectable by karyotyping, gene disruptions, gene fusions, position effects, alterations in topologically associated domains (TADs), and disrupted genomic interactions.

Material and Methods: Six patients presenting phenotypic abnormalities with balanced chromosomal rearrangements identified by karyotyping and the presence of CNVs excluded by chromosomal microarray analysis were investigated using optical genome mapping (OGM) to explore the underlying pathogenic mechanisms. Further analyses of altered TADs and gene-protein interactions were conducted through in silico approaches. Genotype-phenotype correlation studies were performed for all selected patients.

Results: Initial OGM findings revealed potential overlapping mechanisms with gene disruptions identified in five of the six patients, CNVs in two of the six, and TAD disturbances affecting candidate genes in three of the six. Disruption of the *TCF4* gene was found in a patient with t(14;18)(q13.2;q21.2), representing one of the few documented translocations associated with Pitt-Hopkins syndrome, which is characterized by motor delay, stereotypic movements, breathing abnormalities, and other clinical features. In a patient with t(5;17)(p13.2;q11.2), disruption of the *PRLR* gene, along with TAD impairment involving the *NF1* gene, may be associated with phenotypes such as osteopenia, amenorrhea, and microcephaly, among others.

Conclusion: OGM demonstrated significant value in uncovering pathogenic mechanisms, advancing etiological understanding, guiding diagnoses, and shortening the diagnostic odyssey for patients with balanced chromosomal rearrangements.

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P14.018.B Long-Read Sequencing for Structural Variant Detection and Analysis: Mechanism Characterization

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Background: Accurate characterization of Structural Variants (SVs) is crucial for precise diagnosis. While conventional and molecular cytogenetic techniques provide partial insights, they have limitations in resolving SVs within complex rearrangements and repetitive regions. These challenges can be addressed by third-generation sequencing technologies.

Material and Methods: We analyzed ten patients with SVs previously identified by CGH-array and classified as variants of uncertain significance or likely pathogenic based on clinical presentation. Long-read DNA sequencing (LRS) using Nanopore technology was employed to enhance the characterization of these variants and to develop hypotheses regarding their underlying mechanisms of formation.

Results: The analysis identified the following structural variants: 1 DUP-TRP/INV-DUP, 1 DUP-NML-DUP/INV, 6 tandem duplications, 1 tandem triplication, and 1 inversion. LRS enabled precise breakpoint characterization and provided insights into their underlying mechanisms of formation. Among the tandem duplications, five were likely mediated by Fork Stalling and Template Switching or Microhomology-Mediated Break-Induced Replication, both associated with microhomologies. The remaining tandem copy-gain variants were likely mediated by the classical Non-Homologous End Joining mechanism, as evidenced by insertions at the breakpoints. Both complex rearrangements appeared to involve two distinct mechanisms. Lastly, the inversion was likely mediated by Non-Allelic Homologous Recombination, with segmental duplications observed at its breakpoints.

Conclusion: LRS enables the precise detection and reconstruction of complex variants that were not fully characterized by CGH-array, as well as the identification of previously undetected variants. It provides base-pair resolution of breakpoint sequences for all structural variants, facilitating the determination of the specific mechanisms underlying their formation.

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P14.019.C Not just Turner, rare X chromosome rearrangements causing infertility

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Background: Premature ovarian failure (POF) is heterogeneous and could be caused by a variety of etiologies. Here, we present three patients diagnosed with POF and rare rearrangements on chromosome X.

Material and Methods: We clinically evaluated three patients with POF and performed chromosomal microarray (CMA) and Karyotype.

Results: Patient#1 was a 26-year-old referred for abnormal fragile X testing suggestive of triple X syndrome. The initial Karyotype was reported as normal, but the CMA revealed a deletion of 30 Mb in Xq25q28 and duplication of 36 Mb in Xp22.33p21. On revision, the duplicated segment was located in the deletion area, thereby masking this finding. On clinical evaluation, the patient was healthy with normal height but had infertility and elevated FSH levels. Patient #2 was a 16-year-old girl with secondary amenorrhea and increased levels of FSH. The Karyotype and CMA revealed a large de novo 54 Mb deletion on Xq13.2q25 that includes *MECP2*. She was healthy with a normal height and physical examination but with suspected borderline intelligence. Patient #3 was 35 years old with severe oocyte quality after ovum aspiration. CMA revealed a pathogenic 110 kb deletion at Xq27.3 including FMR1 and FMR1-AS genes. She had short stature with suspected borderline intelligence.

Conclusion: We describe ultra-rare chromosome X rearrangements associated with apparently isolated POF. We found scarce data in the literature on these abnormalities. A full medical history and physical examination together with Karyotype and CMA, should be performed in such cases to fully understand the phenotype and genetic diagnosis.

C.O.I. Disclosure:

A.F. Khalaileh: None.

P14.020.D Supernumerary heterochromatic marker in fetus with balanced translocation 11,15

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Background: Small supernumerary marker chromosomes (sSMCs) are abnormal chromosomes with unclear origins, often not detectable through traditional karyotyping. Techniques like aCGH and FISH provide more precise characterization of sSMCs, identifying their chromosome origin and gene content. However, aCGH may have limitations in detecting balanced translocations and regions with repetitive sequences, complicating data interpretation.

Material and Methods: We present a case of a 35-year-old pregnant woman with a history of recurrent miscarriages, carrying a balanced translocation between chromosomes 11 and 15. Chorionic villus sampling (CVS) was performed, and aCGH was used to analyze fetal DNA. Short tandem repeat (STR) markers were analyzed to exclude Uniparental Disomy (UPD). Traditional karyotyping and FISH were also employed to assess the fetal chromosomal profile.

Results: aCGH revealed a female genomic profile without chromosome rearrangements. STR analysis excluded UPD on chromosomes 11 and 15. Karyotyping identified a 47,XX,+mar,t(11;15)(q21;q26)mat karyotype, confirming the presence of a sSMC and the maternal translocation. FISH analysis localized the sSMC to the pericentromeric region of chromosome 15, excluding involvement of the Prader-Willi/Angelman syndrome critical region.

Conclusion: The sSMC detected in the CVS sample was found to be heterochromatic, which explains why it was not detected by aCGH. This case represents a rare occurrence of a sSMC originating from one of the chromosomes involved in a balanced translocation. The origin of the sSMC in this fetus may be linked to a chromosomal rescue mechanism associated with the maternal translocation.

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P14.021.A The value of regions of heterozygocity metrics for predicting morbidity: insights from a large cohort of chromosomal microarray analyses

Lena Sagi-Dain[1], *Michal Levy*[2], *Reut Matar*[2], *Sarit Kahana*[2], *Ifaat Agmon-Fishman*[2], *Cochava Klein*[2], *Merav Gurevitch*[2], *Lina Basel-Salmon*[2], *Idit Maya*[3]

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Background: This study aimed to define the optimal Regions of Homozygosity (ROH) size cut-offs for prediction of morbidity, based on a large cohort of Chromosomal Microarray Analyses (CMA).

Material and Methods: This retrospective cohort study analyzed data from 13,483 CMA tests conducted between April 2017 and December 2023. Receiver operating characteristic (ROC) curves were generated, and area under the curve (AUC) was used to assess the predictive capability of total ROH percentage (TRPS), ROH number and ROH segment size in distinguishing between healthy ($n = 6,196$) and affected ($n = 6,839$) cohorts.

Results: ROH segments were identified in 13,035 samples (96.7%), encompassing 66,710 ROH segments. Significant differences in TRPS and ROH segment size were observed between healthy and affected cohorts ($p = 0.012$ and $p < 0.001$, respectively). In addition, both telomeric and interstitial ROH segments exhibited greater sizes in the affected cohort. However, no clinically significant thresholds could be established based on ROC curves for TRPS and ROH number per sample, as well as for ROH size (AUC 0.64, 0.55 and 0.62, respectively). The same was noted for telomeric vs. interstitial locations, various origins, as well for several subcategories of TRPS.

Conclusion: This study highlights the complexity of ROH interpretation and emphasizes the importance of tailored reporting strategies in clinical practice, particularly in populations with notable consanguinity.

Table 1. Regions of Homozygosity (ROH) and total ROH percentage (TRPS) metrics - comparison between healthy and affected cohorts.

	Healthy (6196 samples)	Affected (6839 samples)	p	AUC
TRPS per sample (%)	0.59 (0.37–0.91)	0.60 (0.36–1.00)	0.01163	0.640
Number of ROH segments per sample	4 (3–6)	4 (2–6)	0.4404	0.552
ROH segment size (Mb)	3.7 (3.3–4.6), n = 30,656 mean 4.6 ± 3.5, max 86	3.9 (3.3–5.5), n = 36,054 mean 6.0 ± 6.3, max 116	<0.0001	0.618
ROH segment size by location (Mb)				
Telomeric ROH	4.0 (3.3–5.7), n = 1,039	5.2 (3.7–9.2), n = 2,254	<0.0001	0.471
Interstitial ROH	3.7 (3.3–4.6), n = 29,617	3.9 (3.3–5.3), n = 33,800	<0.0001	0.630
ROH segment size by origin (Mb)				
Ashkenazi Jewish	3.7 (3.3–4.7), n = 5,959	3.7 (3.3–4.7), n = 4,810	0.608	0.703
Other Jewish	4.2 (3.4–6.0), n = 3,556	4.2 (3.4–6.1), n = 4,633	0.967	0.631
Arab/ Druze	5.3 (3.7–10.5), n = 2,137	6.2 (3.9–12.4), n = 9,356	<0.0001	0.522
ROH segment size by TRPS (Mb)				
TRPS < 1%	3.6 (3.2–4.2), n = 18,567	3.6 (3.2–4.2), n = 16,790	0.070	0.760
TRPS 1–1.99%	3.8 (3.3–4.7), n = 8,494	3.8 (3.3–4.9), n = 7,827	<0.0001	0.672

	Healthy (6196 samples)	Affected (6839 samples)	p	AUC
TRPS 2–2.99%	4.5 (3.6–6.5), n = 1,000	4.4 (3.5–6.5), n = 2,019	0.475	0.616
TRPS 3–4.99%	5.0 (3.7–8.4), n = 876	4.8 (3.7–8.5), n = 1,945	0.294	0.597
TRPS ≥ 5%	7.0 (4.3–13.7), n = 1,719	7.8 (4.5–14.9), n = 7,473	0.002	0.517

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P14.022.B Optical genome mapping and long-read sequencing unravel the mechanisms and phenotypic implications of constitutional complex chromosomal rearrangements

Bruna Burssed[1,2], *Bart van der Sanden*[2], *Kornelia Neveling*[2], *Wolfram Hops*[2], *Eveline Kamping*[2], *Ronald van Beek*[2], *Amber den Ouden*[2], *Ronny Derks*[2], *Raoul Timmermans*[2], *Eduardo Perrone*[1], *Marco Antonio Ramos*[1], *Fernanda Teixeira Bellucco*[1], *Alexander Hoischen*[2,3], *Maria Isabel Melaragno*[1]

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Background: Complex chromosomal rearrangements (CCRs) are structural variants (SVs) that involve at least three breakpoints. Their complete characterization allows for mechanism of formation inference and phenotype correlation.

Methods: Six patients had their constitutional CCRs fully characterized through karyotyping, chromosomal microarray, optical genome mapping (OGM), and 10-fold coverage long-read genome sequencing (LRS).

Results: Three patients presented multiple-chromosome CCRs while the other three involved a single chromosome. The fewest breakpoints (four) were detected in a patient with duplication-inverted/triplication-duplication in chromosome 2, while the most (47) were identified in a patient with a six-chromosome CCR. Given its higher resolution, LRS could detect more breakpoints (136 versus 116) than OGM, which presented a breakpoint uncertainty of ~5,853 base pairs on average. OGM's ultra-long reads and de novo assembly maps, however, achieved the best contiguity of complex SVs and guided the visual inspection of chimeric LRS reads, which especially proved valuable in the ~60% of the CCR junctions for which no automatic SV call was made. Two patients with breakpoints in previously unmapped regions, such as the acrocentric chromosomes' short arms, were resolved using the T2T reference genome. Non-homologous end-joining and chromoplexy were the most prevalent mechanisms of formation since most junctions exhibited break-end joining and insertions. Gene dosage alterations and regulatory context disruptions were associated with the patients' phenotypes.

Conclusion: The combination of OGM and LRS allowed us to assess CCRs at nucleotide resolution for all cases, helping to determine the mechanism of formation of complex SVs and to correlate them with patients' phenotypes.

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P14.024.D Cytogenomic characterization of karyotypes with additional autosomal material and the genomic features around breakpoints

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Background: Chromosomal rearrangements involving additional material in individuals with phenotypic alterations usually result in partial trisomy, often accompanied by partial monosomy.

Objective: To characterize chromosomal rearrangements and analyze genomic characteristics in the breakpoint regions in 31 patients with additional material on an autosomal chromosome.

Methods: Different tests were performed to characterize these patients, such as karyotyping, chromosomal microarray analysis (CMA), and fluorescent in situ hybridization (FISH). In silico analyses were used to evaluate A/B chromosomal compartments, segmental duplications, and repetitive elements at breakpoints.

Results: The 31 rearrangements resulted in 47 copy number variations (CNVs) and a range of structural aberrations were identified, including six tandem duplications, 19 derivative chromosomes, two intrachromosomal rearrangements, one recombinant, two dicentric chromosomes, and one triplication. A deleted segment was associated with the duplication in 16 of the 19 patients with derivative chromosomes from translocation. Among the trios whose chromosome rearrangement origin could be investigated, 54,5% were de novo, 31,9% were maternally inherited, and 13,6% were paternally inherited from balanced translocations or inversion. Breakpoint analysis revealed that 22 were in the A compartment (euchromatin), 25 were in the B compartment (heterochromatin), and five were in an undefined compartment. Additionally, 14 patients had breakpoints in regions of segmental duplications and repeat elements.

Conclusion: Through various cytogenomic methodologies, different chromosomal rearrangements were described in patients with additional material, and it was possible to highlight the effects of parental origin in their rearrangements, also the genomic architecture of breakpoint regions could be elucidated and better characterized.

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P14.025.A CNVs in the imprinted 11p15.5 regions: Frequency, anticipation, heterogeneity and unpredictability

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Background: Disturbances of the imprinting centres IC1 and IC2 in 11p15.5 are associated with Beckwith-Wiedemann and Silver-Russell syndrome (BWS, SRS). Among the different molecular

disturbances are copy number variants (CNVs), which represent a challenge for clinical management and genetic counselling, due to different sizes and variable functional consequences. Advances of diagnostic methods have improved the sensitivity to identify and characterise these CNVs, but their interpretation is still difficult.

Material and Methods: In a cohort of more than 2000 patients referred for diagnostic BWS or SRS testing, the diagnostic yield for the key molecular disturbances was determined by MS-MLPA. Additionally, the contribution of CNVs in 11p15.5 to the spectrum of variants was delineated. Molecular methods were used to precisely determine their sizes and contents The obtained data were compared with cases from an extensive literature and database search.

Results: The detection rate in the cohort was ~20%, CNVs contributed to ~2% of the cases with a positive testing result. In addition to the common 11p15.5 CNVs comprising both ICs and additional genes, smaller CNVs affecting only one IC were identified both in the cohort and in the literature. The phenotypes of the CNV carriers differed markedly, ranging from patients with BWS or SRS features to unaffected.

Conclusion: The heterogeneous clinical outcome of carriers of CNVs affecting 11p15.5 represent a challenge in respect to prognosis, clinical management and genetic counselling. A decision tree to address these aspects in patients with 11p15.5 disturbances will be presented.

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P14.026.B Hunting for the Y chromosome in a 45,X boy - when cytogenetics fails, novel approaches come to aid

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Background: An 8-year-old phenotypic male presented with coarctation of the aorta, short stature, and dysmorphic features. Physical examination showed descended testes and glanular hypospadias. Cytogenetic analysis of peripheral blood lymphocytes revealed a 45,X[11].nuc ish(SRY-)[203] karyotype, and skin fibroblasts showed 45,X[11].ish Xp11.1(DXZ1x1),(DYZ1-),(SRY-)[12]. To clarify the mechanism of male sex differentiation and assess gonadoblastoma risk, we employed highly sensitive methods to detect Y chromosome material in various tissues.

Material and Methods: DNA was isolated from peripheral blood, buccal swabs and urine. ddPCR was designed to target *AMELX* and *AMELY* (blood, buccal swabs, urine) and *SRY* in a separate assay (blood, buccal swabs) with the QX200 Droplet Digital PCR System (Bio-Rad). Data analysis used QuantaSoft. STR analysis (blood, buccal swabs, urine) employed the PowerPlex® Y23 System (Promega) for 23 Y chromosome loci with the detection of products by ABI 3130 Genetic Analyzer (Applied Biosystems) and analysis in GeneMapper ID.

Results: ddPCR revealed presence of the *SRY* gene in the buccal swab, but not in the blood, and *AMELY/AMELX* signal ratios of 0.01 (blood), 0.60 (buccal swab), 0.15 (urine). STR analysis confirmed the presence of 18/23 Y chromosome loci in the buccal swab and urine, but revealed no Y chromosome signal in the blood.

Conclusion: Presence of Y chromosome comprising *SRY* gene was confirmed at varying levels of mosaicism across tissues, explaining the male phenotype. The absence of 5 STR loci at Yq11.22 (AZFc), suggestive of partial Y loss, points to the driving genomic aberration at the origin of the chromosomal abnormality.

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P14.027.C The integration of short and long read genome sequencing alongside optical mapping in the discovery of a mitochondrial insertion in the gene SLC6A8

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Background: Short fragment genome sequencing (sfGS) is the gold standard for molecular diagnosis of neurodevelopmental disorders. However, the identification of certain structural variants (SV) can be difficult to identify using this technique. In such cases, long fragment genome sequencing (lfGS) can be used as an alternative, which facilitates de novo genome assembly, SV identification and haplotype phasing. Optical genome mapping (OGM) can also be utilized as it identifies SV and constructs high resolution genome maps.

Material and Methods: We performed sfGS abd lfGS alongside OGM in a male proband that presents with isolated neurodevelopmental delay.

Results: The initial analyses of sfGS did not identify any variant that could explain the patient's phenotype. An MRI spectroscopy was subsequently performed which showed the absence of a creatine peak, and was in favour of a creatine transporter deficiency due to loss of function of the *SLC6A8* gene. Direct visualization of the sfGS sequences with IGV software led to the suspicion of intra-exonic chromosomal rearrangement involving this gene, present in the hemizygous state in the patient and in

mosaic form in his mother. In combining lfGS and OGM, we confirmed the presence of a mitochondrial insertion in the *SLC6A8* gene. Currently, mitochondrial DNA insertions in nuclear DNA have rarely been reported as being responsible for human constitutional pathologies.

Conclusion: This finding illustrated the importance of combining genome sequencing techniques to achieve accurate molecular diagnoses where previous analyses remained non-conclusive and the rarely observed possibility of mitochondrial DNA insertion as a novel pathological mechanism.

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P14.028.D Split-read analysis of short-read genome sequencing data identifies a de novo congenital chromosome 13 chromothripsis event contributing to bilateral retinoblastoma

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Chromothripsis is an extreme type of complex chromosomal rearrangement that arises when a chromosome is shattered in many fragments in a single, catastrophic event and is then repaired in a random pattern not directed by homology. We performed short-read (150-bp) whole-genome sequencing on a 33-year-old man who had had bilateral retinoblastoma as a child and was planning to have children. Analysing the *RB1* gene we discovered a large 1.3-Mb deletion encompassing the whole gene. Interestingly, when we analysed the split-reads at the apparent breakpoints flanking *RB1* we established that the reads mapped reliably at distant coordinates along the long arm of chromosome 13. We went on to perform sequence analysis of all split-reads around potential breakpoints along chromosome 13. In total, we detected 15 high-confidence breakpoints that had led to four large deletions and multiple intrachromosomal translocations in inverted or non-inverted orientation that most likely arose after chromothripsis. MLPA analysis confirmed the hemizygous deletion of *RB1*, and long-read sequencing validated all the breakpoints detected. The patient was found to carry the low-penetrance pathogenic variant Ile157Thr in *CHEK2* that could have potentially contributed to the rearrangement event, as has been suggested for other DNA repair genes like *TP53*. Preimplantation genetic testing was performed on one embryo based on rare informative SNPs located on the functional *RB1* copy of the father. This embryo had not inherited the rearranged chromosome 13 but was unfortunately chaotic. This case illustrates how application of different bioinformatic algorithms could detect even complex structural variation resulting from chromothripsis.

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P14.029.A Pathogenic complex genomic rearrangements in rare diseases: mechanisms, clinical implications, and diagnostic strategies

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Background: Complex genomic rearrangements (CGRs) are underrecognized contributors to genetic disorders due to the limitations of routine diagnostic methods. These structural variants, often involving multiple breakpoint junctions alongside copy-number changes and inversions, can disrupt gene function, alter regulatory landscapes, and contribute to disease phenotypes. However, their detection and characterization require advanced sequencing technologies capable of resolving structural complexity at nucleotide resolution

Material and Methods: In this study, we analyzed in house data from 64 CGRs, including both previously published (n = 48) and newly identified cases (n = 16), encompassing 536 breakpoint junctions (BPJs) mapped at single-nucleotide resolution. Genome sequencing data were generated using short-read (n = 52) and long-read (n = 33) technology. The CGRs were classified into two groups: intrachromosomal events (n = 53) and complex translocations involving multiple chromosomes (n = 11).

Results: The distribution of BPJs between the groups were 255 BPJs in intrachromomal events (range 2–26) and 274 BPJs in complex translocations (range 2–132). Of note, inversions were observed in 78% of the CGRs, with no significant difference between the two groups.

Conclusion: Our findings highlight the need for long-read sequencing, the telomere-to-telomere reference genome to fully resolve CGRs implicated in neurodevelopmental disorders, autism, Alzheimer's disease, and congenital malformations. The high prevalence of inversions in CGRs suggests specific mechanistic pathways driving structural complexity, with implications for mutational processes and disease risk. These insights reinforce the value of advanced sequencing technologies in identifying previously undetected structural variants, pinpointing disease-causing events, and improving rare disease diagnostics and personalized medicine.

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J. eisfeldt: None. **A. Lindstrand:** None.

P14.030.B Can long-read sequencing replace current cytogenetic methods in clinical genetic diagnostics?

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Background: Detection of structural variants and complex chromosomal rearrangements is a challenge with current methods used for molecular diagnostic of genetic disorders. Balanced translocations are one example of structural variants

where traditional cytogenetic methods is the only method used for detection, which have a low resolution. Long-read sequencing in genetic diagnostics holds great potential as it combines the advantages of traditional cytogenetic methods with the base-pair-level resolution of newer molecular methods. This study wants to explore the capability of long-read sequencing to detect structural variants and fine map the involved breakpoints for future use in genetic patient diagnostics.

Material and Methods: To obtain a broad variety of variants both blood samples from patients with known disease-causing variants and Coriell lymphoblastoid cell lines are included in the project. A broad spectrum of variants has been sequenced, including balanced and unbalanced translocations, duplications, deletions, inversions and complex chromosomal rearrangements. Long-read whole genome sequencing was preformed using Oxford Nanopore Technologies PromethION (p24) platform for all samples.

Results: Results from more than 30 samples have demonstrated that long-read sequencing data enables fine mapping of break points that are inaccessible by current methods. However, for some arears of the chromosome there are still challenges to overcome before detecting all variants with long-read sequencing.

Conclusion: The research to date supports the potential future use of nanopore sequencing in genetic patient diagnostics. The next step prior to implementation would be to validate the method, before using the method for patient diagnostic.

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P14.031.C Familial segregation of a 2q23.1 deletion in the *MBD5* Gene: Reevaluation of pathogenicity based on asymptomatic carriers

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Background: The *MBD5* gene, located on chromosome 2q23.1, encodes a protein involved in DNA methylation and chromatin remodeling. Disruption of *MBD5* through heterozygous deletions or point mutations leads to *MBD5*-associated neurodevelopmental disorder (MAND), characterized by autosomal dominant inheritance. MAND manifests with universal intellectual disability, alongside common features such as seizures, dysmorphic traits (short stature, microcephaly), sleep disturbances, ataxia, aggressive behavior, and hyperactivity. *MBD5* deletions are relatively rare, detected in approximately 0.05% of 17,477 individuals undergoing clinical microarray analysis. The majority of these cases are de novo, although rare instances of inheritance from mildly affected or mosaic parents have been described

Material and Methods: We report a family that underwent prenatal diagnosis without a specific medical indication

Results: Chromosomal microarray analysis (CMA) revealed a 172 kb deletion at the 2q23.1 locus encompassing exons 3 and 4 of *MBD5*. Due to the potential clinical risk, the pregnancy was terminated. Subsequent parental testing identified paternal inheritance of the deletion. Family segregation analysis, including ten paternal relatives, revealed two additional asymptomatic carriers (the paternal grandfather and a brother) who harbor the same deletion

Conclusion: Segregation analysis documented three unaffected individuals carrying the 2q23.1 deletion. This evidence suggests that in this family, the deletion may not lead to the severe phenotypic presentation typically associated with *MBD5* deletions, although a pathogenic effect cannot be definitively excluded. Consequently, the variant classification was revised from 'Likely Pathogenic' (LP) to 'Variant of Uncertain Significance' (VOUS)

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P14.032.D Detection of a rare 46,XXX,t(13;14)(q10;q10) karyotype in a patient with primary infertility and recurrent pregnancy loss

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Background: Robertsonian translocations, particularly t(13;14), are associated with reproductive challenges such as infertility and recurrent pregnancy loss. Triple X syndrome (47,XXX) is a sex chromosome aneuploidy that may also impact fertility, though many individuals remain asymptomatic. The co-occurrence of 47,XXX and a Robertsonian translocation is rare, with limited literature on its clinical and reproductive implications.

Material and Methods: A 44-year-old female with 4 years of primary infertility, a history of a terminated distal ectopic pregnancy (cervical), and a failed insemination attempt was referred to the "Fertility Center" Reproductive Center. Cytogenetic analysis was performed using G-banding karyotyping on peripheral blood lymphocytes. Fluorescence in situ hybridization (FISH) was used to confirm the translocation and exclude cryptic rearrangements. Hormonal profiling and ovarian reserve testing were conducted to assess reproductive health. Biological parents were unavailable for study, and the origin of the chromosomal findings could not be determined.

Results: Karyotype analysis revealed a 46,XXX,t(13;14)(q10;q10) chromosomal complement, confirming the presence of a balanced Robertsonian translocation in conjunction with triple X syndrome. FISH analysis validated the translocation breakpoints and ruled out additional structural abnormalities. Hormonal testing indicated diminished ovarian reserve, consistent with the patient's reproductive history.

Conclusion: This case highlights the rare coexistence 47,XXX and a Robertsonian translocation, t(13;14)(q10;q10), and its potential impact on reproductive health. The findings emphasize the importance of comprehensive cytogenetic evaluation in individuals with infertility and recurrent pregnancy loss. Further studies are needed to elucidate the mechanisms underlying the reproductive challenges in such cases.

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P14.033.A Partial trisomy and partial tetrasomy of chromosome 15q in Newborn cases associated with developmental delay, hypotonia, intellectual disability and autistic behavior

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Background: Triplication and tetraplication of chromosome 15q is a rare developmental genetic disorder that may be presented with variable presentation of developmental delay, hypotonia, intellectual disability and autistic behavior.

Material and Methods: Patient's physical examinations, conventional chromosomal analysis, fluorescence in situ hybridization (FISH) analysis and array-CGH were performed as a standard genetic care.

Results: Here, we reported four newborns with various congenital anomalies and autistic spectrum disorder with chromosome abnormality of interstitial duplication (partial Trisomy) of 15q11q13 in two cases verses a unique epilepsy and other findings with an extra supernumerary marker (Partial tetrasomy) chromosome 15 in the two other cases. Subsequently array CGH and FISH analysis were confirmed the de novo finding of triplication and tetraplication imprinting of 15q11-q13 region.

Conclusion: The 15q imprinting syndrome is a rare genetic disorder that may presented with variable unique clinical presentation according to the size of triplication or tetraplication of the Prader-Willi/Angelman Critical Region. Furthermore an extensive genetic analysis with array CGH and FISH may provide a robust diagnostic methodology that improve early diagnosis and better patient counseling.

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P14.034.B Single-molecule long-read sequencing provides fine characterization of 22q12 microdeletions in NF2 patients unravelling specific patho-genetic and -epigenetic mechanisms

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Background: Neurofibromatosis type 2 (NF2) is an autosomal dominant tumor-prone disorder, mainly due to point mutations or intragenic deletions of the tumor suppressor gene *NF2*. Rarely, 22q12 microdeletions have been identified in NF2 patients showing variable phenotypes. Due to the lack of hot spot recombination, few of them have been genetically characterized. We studied two 22q12 microdeletions by a new approach identifying the role of patho-genetic/epigenetic mechanisms to the patients' complex phenotype.

Material and Methods: We carried out aCGH analysis to ascertain the deletion extent, Nanopore long-read sequencing (LRS) to identify the deletion breakpoints, Sanger Sequencing to validate the junction fragment, and in silico analysis of gene content and breakpoints' sequences. To study a possible position effect generated by chromatin alteration, we performed in silico analysis of 22q12 TADs and regulatory elements, expression analysis by qPCR on 12 genes flanking patients' deletions, and LRS DNA methylation study. The role of detected gene variants in the onset of patients' phenotypes was also evaluated.

Results: Patients 568 and 690 show deletions spanning 3.08 and 2.35 Mb, respectively, involving 44–47 genes and 5–6 TADs. The position effect on specific flanking genes was detected by qPCR, according to abnormal DNA methylation profiles. Genotype-phenotype correlation studies revealed possible associations between *AP1B1* gene haploinsufficiency and cognitive impairment in PT568, and the *EVC* pathogenic variant and congenital heart defects in PT690.

Conclusion: The LRS approach is an effective advancement for studies of pathomechanisms in NF2 microdeletion patients, improving the identification of prognostic markers, counselling, and patient management.

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P14.035.C De novo deletion in *NCKAP1* and monosomy 18 mosaicism in a 6-year-old boy with developmental delay and autism

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Background: Loss-of-function variants in *NCKAP1* have been associated with complex neurodevelopmental delay. Two cases of monosomy 18 (−18) in mosaic form have been reported, one with severe neurological symptoms [1], and another with a cleft lip and palate and mild behavioural problems [2]. We report a new case of a 6-year-old boy with developmental delay, autism, and intellectual disability, with a pathogenic variant in *NCKAP1* and mosaic -18.

Materials and Methods: The proband was born at term to non-consanguineous parents. Global developmental delay, mild intellectual disability, and autism were diagnosed at age four. Sleep EEG showed asymptomatic abnormalities. On examination, he had a long face with downslanted palpebral fissures, anteverted nares, and low-set ears as well as hypertrichosis with strong eyebrows and long eyelashes. Cytogenetic, FISH, array-CGH, and exome analysis were performed.

Results: GTG-banding of two different cultures showed the karyotype mos 45,XY,-18[5]/46,XY[17], confirmed by FISH. Mosaic -18 was not detected by array-CGH or FISH analysis of saliva. Exome sequencing revealed the de novo variant c.3161_3164del; p.(Arg1054Leufs*13) in *NCKAP1*.

Conclusion: This case enriches the sparse landscape of *NCKAP1*-associated developmental delay and mosaic -18. So far, several patients with truncating variants in *NCKAP1* have been reported with neurodevelopmental and behavioral symptomatology consistent with this case. Two previous cases of mosaic -18 have been reported, the phenotypes of which overlap with our case. While this patient's phenotype can be primarily attributed to the *NCKAP1* variant, the significance of the -18 mosaicism detected remains unclear and further investigations are ongoing.

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P14.036.D Effects of prolonged exposure to Ionizing radiation on structural variants in the human germline

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Background: Prolonged paternal exposure to low dose ionizing radiation (IR) is known to cause a wide variety of DNA changes in humans. These range from small changes on the single-bp scale to structural changes, including large Insertions, Deletions and Translocations, mediated by the interaction of IR with DNA and different mechanisms of DNA repair. For our previous works, we recruited a cohort of soldiers of both german armies which were likely exposed to IR during their military service.

Materials and Methods: We analyze de-novo structural variation in whole-genome sequencing (WGS) data from a cohort of 110 offspring of exposed soldiers of both german armies and 1275 children born to parents with no known non-natural exposure to IR. Structural variants (SVs) are detected using an ensemble approach using information from four different structural variant calling algorithms.

Results: DNA repair mechanisms and selection and survivorship biases inherent to the human germline introduce important confounders in our analysis. Only damage to human DNA that is not within a narrow band of large-enough to be inherited by the next generation but small enough to not cause fatal damage to the cell or human embryo is detectable in the trio-WGS data of our cohorts. We aim to find SVs associated with the increased paternal exposure to IR, while addressing the aforementioned and other confounders. Our previous small scale studies suggested increased numbers of balanced Translocations and similar SVs as a potential observable biomarker in the offspring of Radar soldiers.

C.O.I. Disclosure:

F. Brand: None. **A. Knaus:** None. **P. Krawitz:** None.

P14.037.A Molecular cytogenetic analyses of two cases of constitutional non-supernumerary ring chromosomes: r(2) and r(7)

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Background: Human ring chromosomes (RCs) are rare and unique chromosomal abnormalities, reported in all chromosomes. These RCs are generally classified into complete, incomplete, or complex rings.

Here, we report and discuss the results of chromosomal microarray (CMA) and fluorescence in situ hybridization (FISH) performed on two cases of constitutional non-supernumerary RCs, detected by G-banding.

Case 1: r(2)(p25.3q37.3)

Previous subtelomeric FISH showed signals at both 2pter and 2qter on the r(2), leading to its initial classification as a 'complete ring' without deletions at either end (Kosho et al., 2005). However, subsequent CMA analysis revealed a ~200 kb copy number loss at 2pter. We then confirmed the presence of the deletion in the r(2) by metaphase FISH using a BAC clone that mapped to the deleted region.

Case 2: r(7)(p22q36)

CMA analysis revealed a 7pter loss (~1.0 Mb) and a 7qter gain (~2.4 Mb). Metaphase FISH using selected BAC probes specific to each CNV region confirmed the presence of both CNVs in the r(7). To further investigate whether either parent was a carrier of the pericentric inversion of chromosome 7, metaphase FISH analyses were performed with selected BAC probes and the mBAND7 probe, revealing normal chromosomes 7 patterns in both parents.

Conclusion: CMA analysis is effective in detecting CNVs, particularly microduplications, which are difficult to identify by G-banding. However, CMA cannot specify which homologous chromosome carries each CNV. Metaphase FISH, performed after CNV detection with appropriate probe selection, is an essential method to identify chromosomal rearrangements associated with one or more CNVs.

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P14.038.B Heterochromatin Dynamics: Unraveling its Role in Phenotypic Modulation

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Background: We report the case of a 40-year-old male patient presenting with oligo-astheno-teratozoospermia which led to the prescription of cytogenetic analyses.

Material and Methods: A constitutional karyotype was performed. This analysis was complemented by chromosomal microarray analysis (CMA) and fluorescence in situ hybridization (FISH).

Results: The karyotype revealed an abnormal chromosome 7, with a long arm larger than its homolog, suggesting an insertion or duplication at 7q11. CMA subsequently identified a 22.8 Mb copy gain in the 2q11.1q14.1 region. This CNV has never been previously reported in literature. FISH analysis demonstrated a direct insertion of the duplicated 2q segment into the centromere of one chromosome 7. The optical mapping of the genome found the duplication but did not show its centromeric insertion as expected. This 22 Mb (7;2) insertion caused no significant phenotypic abnormalities in the patient, except for reproductive impairments. Our hypothesis to explain this attenuated phenotype is that the inserted chromosomal segment may be inactivated by the pericentromeric heterochromatin of chromosome 7. To validate this heterochromatinization phenomenon, we are employing various techniques, including immunofluorescence staining with antibodies against trimethylated histone H3, heterochromatin protein 1, 5-methylcytosine and the centromeric marker CENPA. We also performed RNA sequencing to refine the expression profiling of the inserted region.

Conclusion: If our hypothesis regarding heterochromatin-mediated inactivation is confirmed, this case would illustrate the pivotal role of heterochromatin in the epigenetic regulation of gene expression. It would also emphasize its capacity to mediate heterochromatinization of large chromosomal segments.

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P14.039.C Defining breakpoint regions with Optical Genome Mapping (OGM)

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KK Women's and Children's Hospital, Singapore, Singapore.

Background: A 11-month-old baby was investigated for global developmental delay, hypotonia, short stature and strabismus. Chromosomal Microarray Analysis (CMA) was performed and as part of ongoing validation studies, Optical Genome Mapping (OGM) was also done.

Material and Methods: CMA was performed using Agilent Technologies SurePrint G3 ISCA CGH+SNP 4 × 180K and analyzed with Cytogenomics software. Extraction of ultra-high-molecular-weight genomic DNA and DNA labelling was carried out as per Bionano's instructions. Analysis was performed using Bionano Access, comparing the results of the sample with the GRCh38/hg38 reference genome map.

Results: CMA showed a female profile with a gain of 0.506 Mb at 6q24.3, arr[GRCh38] 6q24.3(145792859-146299000)x3. The gain results in the partial gene gain of 1 OMIM gene associated with phenotype, *GRM1*. OGM found that this gain was directly inserted into *GRM1*, disrupting the gene.

Conclusion: Heterozygous pathogenic variants in *GRM1* are associated with Spinocerebellar ataxia 44 (MIM: 617691), and biallelic pathogenic variants in *GRM1* are associated with Spinocerebellar ataxia autosomal recessive 13 (MIM:614831). CMA analysis cannot determine if the duplicated segment of *GRM1* is located within the gene (causing gene disruption) or inserted elsewhere in the genome. However, OGM revealed a direct insertion of the gain into 6q24.3, which is predicted to disrupt the gene *GRM1*. We will discuss possible implications of this heterozygous *GRM1* disruption in relation to the patient's phenotype. This example illustrates the utility of OGM in defining breakpoint regions and how the integration of OGM into the clinical workflow can help clinicians gain better understanding of structural variants.

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P15 Genomics of rare diseases

P15.001.D Genome screening for mobile element insertions in undiagnosed rare diseases

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Background: Detection and interpretation of point mutations, small deletions, insertions, and even copy number variants are well established in clinical genome sequencing. In contrast, mobile elements insertions (MEIs) are technically difficult to detect and rarely reported. Our objective was to establish a MEI detection tool in routine diagnostics to solve undiagnosed cases and assess its impact on diagnostic yield.

Methods: In this study, we applied the Mobile Element Locator tool (MELT) for the detection of MEIs and screened our in-house database of 5000 clinical genome datasets of undiagnosed rare disease patients. Detected MEIs in coding, splicing and near-splice regions were prioritized for phenotype-based filtering strategies.

Results: The analysis demonstrated that reanalyzing genomes for the detection of MEIs located in exons or near splice sites and with rare allele frequency identified unsolved cases. Disease-causing MEIs, e.g. LINE- and Alu-elements were found in the genes *SLC17A5*, *PTEN* and *CTNNB1*, compatible with the patients phenotype. Following segregation and transcriptomics analysis a firm genetic diagnosis was made for three patients.

Conclusions: MEIs very rarely occur in coding regions of the human genome, however this study shows that the bioinformatic MELT tool can lead to additional diagnosis in routine work, here concerning mental retardation and neurological disorders.

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P15.002.A Solving the Unsolved: The Utility of Long-Read Genome Sequencing in the Care4Rare Canada Cohort

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Background: Long-read genome sequencing (LR-GS) is emerging as a useful tool to identify the genomic causes of unsolved rare diseases (RDs). It can accurately detect a range of genomic variants and overcome some limitations of short-read genome sequencing (SR-GS) including improved structural variant detection and sequencing highly repetitive, highly similar, or low-complexity regions. We therefore aimed to apply LR-GS to identify disease-associated variants in our unsolved RD cohort.

Materials and Methods: Families enrolled in the Care4Rare Canada research project with a suspected monogenic disorder without definitive molecular diagnoses following clinical testing and research SR-GS, or those requiring LR-GS to clarify a previously identified variant(s), were sequenced on the PacBio Sequel IIe or Revio systems. Data was processed using the PacBio Human WGS workflow and in-house pipelines.

Results: To date, 51 families (139 participants) with unsolved RDs have been sequenced and 25 have initial analyses complete. LR-GS revealed a new disease-associated variant in two families (8%), including an intronic retroelement insertion and a novel chromoanasynthesis event. In another four families (16%), LR-GS clarified previously identified variants to establish their association with disease. In one family (4%), a previously identified duplication was revealed to be a more complex structural variant, however this requires further investigation to confirm disease-association. Seventeen families (68%) remain unsolved.

Conclusion: LR-GS contributed to solving 24% of unsolved RDs in our cohort so far. LR-GS was most useful to discover/clarify complex genomic rearrangements, tandem repeat expansions, or other challenging variants for SR-GS (e.g. retroelement insertions), and for variant phasing.

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P15.003.B Stanford Center for Undiagnosed Diseases: a decade of advancing genetic diagnosis and discovery

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Background: Despite the rapid progress in clinical genetic testing, most patients with a suspected rare disease remain undiagnosed after extensive medical evaluation. The Stanford Center for Undiagnosed Diseases (CUD) was established in 2015 and is a clinical site of the National Institutes of Health supported Undiagnosed Diseases Network. At the CUD, we developed an approach that integrates phenotyping, genomics, other omics assays, and model organism studies to improve diagnosis and care for patients with rare diseases.

Material and Methods: We evaluated patients with undiagnosed diseases who were referred to the CUD between 2015 and 2025. We determined the diagnostic rate and highlighted genetic discoveries facilitated by the program.

Results: A total of 283 patients were evaluated by the CUD, of which 53% were female, 61% were pediatric and 59% were non-Hispanic White. Patients primarily had neurological (42%), musculoskeletal (13%), immunological (12%), and cardiovascular (7%) symptoms. Patients underwent short read exome or genome sequencing (95%), RNA-seq (68%), metabolomics (21%), immunomics (19%) and long read genome sequencing (10%). Overall, 36% of patients (102) received a likely or candidate diagnosis including 97 molecular, 4 clinical, and 1 antibody-mediated phenocopy of a Mendelian disease. Seven patients received multiple molecular diagnoses. We described 22 novel candidate genes/loci, expanded the phenotype or mode of inheritance for 8 known disease genes, and identified 64 novel causative variants.

Conclusion: Here we present a comprehensive and integrated clinical and multi-omic approach to advance diagnosis and discovery, shorten the diagnostic odyssey, and improve care for patients impacted by rare diseases.

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P15.004.C Germline variants in coagulation genes underlying hereditary unclassified bleeding disorders

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Background: Hereditary bleeding disorders are a rare and heterogeneous group of diseases characterized by genetic defects that impair normal hemostasis. Despite bleeding tendency about 30% of patients present normal hemostatic tests with no identifiable cause, remaining lastingly undiagnosed, sometimes with severe and even fatal consequences. The identification of genetic variants in these unclassified bleeding disorders (UBD) can be essential for providing patients with accurate diagnosis and better disease management, as well as for uncovering new pathophysiological mechanisms.

Material and Methods: Sixteen families reporting a history of UBD (thirty-two affected and fifteen healthy members) underwent whole-exome sequencing. Germline mutation burden was assessed in 425 genes related to coagulation, platelet activation, and endothelium adhesion, selecting non-synonymous variants, with minor-allele frequency <0.01, and not reported as benign in ClinVar. Phenotype and altered genes relationship was analyzed using mixed effect models.

Results: Patients carried 406 variants falling in coagulation (38%), endothelium (34%) and platelet activation (27%) genes. An increased frequency of variants in coagulation genes was associated with UBD ($p = 0.048$). Most frequently mutated genes were *MADCAM1*, *SH2B2*, *DCT* (84–62%), *IL6ST*, and *COL18A1* (28%). In "core" coagulation genes, variants were detected in *VWF* (16%), *F5*, *FGA* (12%), *FGG* (9%), *F2*, *F7*, *F13A1*, *F13B* (6%). Among altered genes, two metalloproteinases involved in hemostatic balance, *ADAMTS18* and *MMP8*, displayed a suggestive association with phenotype ($p = 0.045$; $p = 0.077$).

Conclusion: UBD patients harbored increasing numbers of variants in coagulation genes compared to healthy family members. Alterations in genes *ADAMTS18* and *MMP8* might also play a role in UBD development.

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P15.005.D Results from Rapid Whole Genome Sequencing (rWGS) in patients presenting with Neonatal and Pediatric Hypotonia

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Background: Whole Genome Sequencing (WGS) represents a complete testing approach to rapidly identify an underlying molecular defect in children with hypotonia by simultaneously assessing multiple variant types. We report the results from clinical WGS at Rady Children's Institute for Genomic Medicine (RCIGM) between 2017 and 2024 in patients with hypotonia at the time of testing.

Material and Methods: WGS was performed at CAP/CLIA RCIGM via PCR-free WGS. Alignment and variant calling were done using the Illumina DRAGEN software. Annotation, variant prioritization and clinical reporting were performed using Fabric Enterprise. Genomic variation assessed spanned SNVs, Indels, CNVs, Aneuploidies, SMN1 and SMN2 copy number, mitochondrial variants, repeat expansions and UPD15.

Results: A total of 5708 patients were evaluated (age median = 50 days). 591 patients had hypotonia or neonatal hypotonia at the time of testing (10.4%), and most underwent rapid or ultra-rapid WGS. A molecular diagnosis was made in 268 cases (45.3%). Recurrent diagnoses included SNVs/Indels affecting GLDC (n = 9), MTM1 (n = 6) KCNQ2 (n = 5), Trisomy 21 (n = 10), CNVs encompassing the 15q11.2q13.1 imprinted region (n = 7), deletions affecting SMN1 (n = 5) and DMD (n = 2). The median TAT for reporting preliminary results was 4 days.

Conclusion: The clinically validated fast WGS pipeline at RCIGM has proven utility in diagnosing patients with hypotonia. This exhaustive testing approach detects a wide range of genomic alterations in a time-effective manner, thus enabling optimal management. Additional refinements and improvements of the technology and breath of identifiable variation will further enhance the diagnostic yield of the RCIGM WGS pipeline in patients with hypotonia.

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P15.006.A Single-Cell RNA Sequencing Demonstrates Specific Sex Hormone Responses of Alveolar Type 2 Epithelial Cells to Profibrotic Exposure

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Background: Pulmonary fibrosis (PF) is a progressive, fatal lung disease characterised by tissue scarring. A recent mouse study highlights a potential protective role of estrogen against PF, suggesting that sex hormones influence the development and progression of PF.

Material and Methods: Here we set out to detail sex hormone responses of alveolar type 2 epithelial cells to profibrotic exposure, using scRNA approach. We generated AT2 cells from induced pluripotent stemcells (iAT2) to examine the effects of sex hormones on gene expression using single-cell RNA sequencing. iAT2 cells were treated with estrogen and testosterone for 14 days, with subsequent profibrotic cocktail (PFC) exposure in the final 72 hours. Single live cells were encapsulated using a 10X-Genomics Core machine and RNA libraries were generated, followed by sequencing. scRNA-seq analysis was conducted using Seurat after identifying the highest-quality cells. Dimensionality reduction techniques were employed to visualise clustering, followed by cluster annotation and pathway enrichment analysis. Heatmaps, violin plots, and feature plots were also generated.

Results: Cluster annotation revealed that estrogen-treated cell clusters exhibited senescence markers following PFC-exposure, whereas testosterone-treated cell clusters demonstrated TGF-β activation and aberrant-basaloid markers. RNA velocity indicated a transition towards aberrant basaloid cells from other clusters, including the TGF-β cluster. Overall, these analysis suggests that testosterone is linked to a potential transition to recognised profibrotic cell states after PFC-exposure.

Conclusion: Our findings suggest distinct roles of sex hormones in modulating AT2 cells, offering insights into understanding PF and its treatment. However, additional laboratory validation is necessary to confirm these observations.

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P15.007.B Reclassification of genomic duplication variants of uncertain significance using nanopore adaptive sampling

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Background: Genomic duplications classified as variants of uncertain significance (VUS) pose a challenge in clinical diagnostics due to their unclear pathogenicity. Short-read sequencing can detect copy number variations (CNVs) but lacks the resolution to define breakpoints accurately. This study aimed to evaluate the feasibility and diagnostic yield of Oxford Nanopore Technologies (ONT) adaptive sampling (AS) for resolving VUS duplications.

Material and Methods: A cohort of 49 patients with VUS duplications identified through short-read sequencing was analyzed using ONT AS. The protocol was optimized to improve sequencing accuracy and coverage, including DNA input and library preparation adjustments. Long-read sequencing data were assessed for breakpoints, reading frame integrity, and variant classification according to ACMG/AMP guidelines.

Results: Tandem duplications were detected in 28 samples (57%), with 7 reclassified as likely pathogenic (18%) or pathogenic (7%), resulting in a 25% reclassification rate. The remaining 75% of cases retained their VUS classification due to insufficient supporting evidence. The optimized protocol improved sequencing efficiency, achieving higher coverage (>24X) and more reliable variant detection.

Conclusion: ONT AS is a feasible approach for detecting structural variants and reclassifying VUS duplications, offering improved resolution for clinical variant interpretation. Its bioinformatics-driven, targeted sequencing strategy eliminates the need for specialized library preparation for specific genes, making it scalable, automatable, and adaptable for routine diagnostics. While additional validation is needed, ONT AS presents a cost-effective and flexible solution for integrating long-read sequencing into clinical practice, ultimately enhancing genetic analysis and patient care.

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P15.008.C Diagnostic yields of exome sequencing, short-read genome sequencing, and long-read genome sequencing in neurofibromatosis type 1

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Background: Neurofibromatosis Type 1 (NF1) is primarily diagnosed using NIH criteria, however molecular testing is increasingly important. The diagnostic yields of exome sequencing (ES), short-read genome sequencing (SR-GS), and long-read genome sequencing (LR-GS) remain unclear due to NF1's complexity.

Material and Methods: Patients meeting NIH criteria for NF1 were recruited from King Chulalongkorn Memorial Hospital between 2019 and 2023. A stepwise sequencing approach was utilized, beginning with ES on the Illumina NovaSeq 6000 platform. For unresolved cases, SR-GS was performed using the BGI DNBSEQ-T7 platform, followed by LR-GS on the PacBio HiFi platform. Variants were classified according to ACMG/AMP guidelines.

Results: Among 94 patients (87 families), ES identified 71 (likely) pathogenic *NF1* variants in 77 patients (71 families) (82%; 71/87), including nonsense (41%, 29/71), frameshift (30%, 21/71), splice-site (17%, 12/71), missense (7%, 5/71), large deletions (4%, 3/71), and large duplications (1%, 1/71). In unresolved cases, SR-GS detected one deep intronic variant in 1 patient (1 family) (1.2%; 1/87), while LR-GS further identified 3 pathogenic structural variants: 2 deletions and 1 inversion, in 3 patients (3 families) (3.5%; 3/87). The combined diagnostic yield was 86% (75/87). The remaining 13 unresolved cases (12 families) may involve somatic mosaicism or variants with uncertain pathogenicity.

Conclusion: ES is an effective molecular diagnostic tool for NF1, with SR-GS and LR-GS serving as a valuable complement to resolve complex cases.

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P15.009.D Diagnostic improvement of rare diseases through Optical Genome Mapping in the SpainUDP Undiagnosed Cases program

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Background: Massive sequencing techniques have enabled unprecedented progress in diagnosing rare diseases. Exome and genome sequencing have great capacity to reliably identify point variants and small insertions and deletions. However, due to the use of short reads, these methodologies do not efficiently identify structural variants (SV) of moderate-large size. The Optical Genome Mapping (OGM) strategy uses long DNA molecules to identify structural variations throughout the genome, hardly detected with short-read sequencing. SpainUDP program has applied OGM and evaluated the diagnostic success of adding this technology in still undiagnosed cases.

Material and Methods: OGM was done in 42 undiagnosed cases analyzed in trios, including a subset of 10 cases performed in the European Solve-RD project. High molecular weight DNA was extracted from blood samples and OGM was made using the Saphyr system (Bionano) and initially analyzed with Access software for detection of de novo SV.

Results: By OGM we could find candidate de novo SV in 10 cases (23%) and managed to definitively diagnose 3 cases (7%), identifying complex de novo SV in patients, including an inversion in *PTH* gene, a retrotransposon insertion in *USP9X* (confirmed with LR-WGS) and a complex case with multiple SV pointing to chromotripsis events. In the other seven cases, we found SV affecting candidate genes, 4 of them associated with syndromes, which are being validated.

Conclusion: By de novo analysis of OGM we expect between 7–14% diagnostic success in cases that remained unexplained. The OGM results highlight the complexity of the structural variation of our genome.

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P15.010.A Targeted long-read sequencing: a cost-effective approach to overcome limitations of short-read sequencing in routine diagnostics (Award Candidate)

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Background: Short-read sequencing (SRS) is the gold standard in genetic diagnostics but remains inconclusive in over 50% of cases. Challenges such as pseudogenes, variants phasing, and structural variations contribute to this diagnostic gap. Whole-genome long-read sequencing (WGLRS) has the potential to overcome these issues but remains costly, data-intensive, and challenging to implement in routine diagnostics. To overcome these limitations, we implemented targeted long-read sequencing (TLRS) as a complementary approach.

Material and Methods: Long-range PCR (amplicons up to 30 kb) was followed by Nanopore sequencing and in-house bioinformatics analysis. We analyzed 200+ cases where SRS was inconclusive but suggested single-gene involvement.

Results: Our analysis demonstrated the utility of TLRS across several challenging scenarios. In 40% of cases TLRS differentiates pathogenic variants from pseudogene artefacts (*PMS2*, *PKD1*, *CYP2D6*) preventing misdiagnoses from short-read sequencing. In 20% of cases, when parental/related samples were not available, it successfully determined the phase of heterozygous variants in recessive genes, allowing accurate interpretation. Another 20% of cases involved the resolution of SRS-suspected copy number variants or indels in complex regions. Nine structural variants were precisely characterized and reclassified, including deletions, inversions, and duplications. Additionally, our approach resolved two WES-negative cases of clinically suspected monogenic disorders (*APRT* compound heterozygosity; *GBA1* variants undetected due to pseudogene interference).

Conclusion: TLRS offers a high-yield and cost-effective alternative to WGLRS. This approach is a powerful complement to SRS, resolving challenges due to pseudogenes, variant phasing, and structural variants characterization. It strengthens diagnostic certainty by providing definitive variant confirmation, exclusion and reclassification.

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P15.011.B UDN Sweden: A Multidisciplinary Approach Integrating Genome and Transcriptome Sequencing to Improve Diagnosis in Undiagnosed Children with Rare Syndromes

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Background: Despite advances in massive parallel sequencing, up to 60% of rare disease patients remain undiagnosed after whole-genome sequencing (WGS), partly due to incomplete phenotype data and challenges in detecting the full spectrum of pathogenic variants. The Undiagnosed Diseases Network (UDN Sweden) unites clinicians, genetic counselors, clinical laboratory geneticists, bioinformaticians, and researchers to improve rare disease diagnostics. Our goal is to enhance genomic technologies in healthcare and ensure equitable precision diagnostics for undiagnosed children with rare syndromes involving intellectual disability and/or congenital malformations.

Methods: Since 2023, 171 children have been enrolled through Wilhelm Foundation and the seven Swedish university hospitals. All remained undiagnosed despite extensive clinical and genetic evaluations. After collecting structured phenotype data using the phenotype tool tip2toe, we performed systematic analyses of short-read WGS (singleton, trio, or quartet), RNA-sequencing (138 participants), and PacBio HiFi long-read WGS (130 participants).

Results: To date, short-read WGS reanalysis has provided definitive genetic diagnoses for eleven individuals, uncovering pathogenic variants in *CKLD5*, *TUBB4A*, *SCN2A, TCF4, RNU4-2, TBCE*, *NF1*, *U2AF2*, and *CUX2*, as well as duplication of *MYT1L* and a 16p12.2 microdeletion. Candidate variants have been identified in three additional cases. Data analysis of the entire cohort is ongoing. In addition, a national hackathon is planned to analyze 12 selected cases.

Conclusion: These preliminary results demonstrate the potential of an integrated phenotype-genomics-transcriptomics approach and a coordinated network of clinical, laboratory, and research expertise to improve diagnostic yield, streamline clinical workflows, and ultimately enhance outcomes for children with rare genetic neurodevelopmental disorders.

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P15.012.C Whole Genome Sequencing as a last-tier test: wouldn't it be better to do it sooner?

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Background: Children with rare and non-critical genetic diseases often face a multi-stage diagnostic strategy that sometimes lasts years. This path has a socio-economic impact on the family and the health system. Achieving a diagnosis is essential for the best clinical management and family counseling. This study evaluated the utility of whole genome sequencing (WGS) as a last-line test.

Material and Methods: We retrospectively analyzed data from 147 outpatients with a suspected genetic condition who underwent trio-WGS after at least array-CGH and multigene panels or whole exome sequencing (WES) in the past two years.

Results: The age of patients covers a wide range (from 0 to 47 years, mean age 10.2 years), although the clinical manifestations were present from birth or early childhood. Most cases had a neurodevelopment disorder (47.6%), followed by malformation syndromes (34%). Most patients (64.1%) had two or more tests performed before WGS. Trio-WGS added a genetic diagnosis to 27.2% of patients. In 13 cases, WES has been previously performed. Three cases had a double diagnosis, causing a compound phenotype. Considering the type of identified variants, ranging from deep intronic variants to complex rearrangements, 30.4% (17/56) would have been missed with other techniques, including WES.

Conclusion: In our experience, genome sequencing has been a resolutive technique for almost a third of cases. These data highlight that using trio-WGS as a first-tier test would shorten the time for a diagnosis, reducing the diagnostic odyssey and health system costs and improving the patients' management.

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P15.013.D Accurate Phasing and Imputation of Rare Variants in Diverse Chinese Subpopulations Using 22,374 Deeply Sequenced Genomes

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Background: Reference panels are critical for improving genotype imputation accuracy in underrepresented populations, yet existing resources often lack diversity in non-European and non-Han Chinese ethnic groups. Addressing this gap is essential for advancing equitable genomic research and precision medicine initiatives.

Material and Methods: The GSRD-100K^{WCH} reference panel was constructed using high-coverage whole-genome sequencing (WGS) data from 22,374 individuals, including 17,514 Han Chinese and 4,860 individuals from 45 non-Han Chinese ethnic groups. Imputation performance was evaluated using simulated and

empirical HGDP array datasets, benchmarking against leading reference panels (e.g., TOPMed). Metrics included precision, sensitivity, aggregate R^2 (genotype concordance), and non-reference discordance (NRD) rates.

Results: The panel comprises 321,365,014 variants, with 34,016,043 (10.58%) unique to non-Han populations. In simulated analyses, GSRD-100K^{WCH} imputed 72.56% more variants than comparator panels (excluding TOPMed), 49.79% of which were panel-specific. It achieved high precision (0.9831) and sensitivity (0.9802), with improved aggregate R^2 and reduced NRD across Chinese subpopulations. While performance was robust in most groups, slight accuracy reductions occurred in admixed populations (e.g., Turkic and Indo-Iranian groups). The panel also outperformed existing resources in imputing Asian-relevant GWAS and polygenic score (PGS) catalog variants.

Conclusion: The GSRD-100K^{WCH} reference panel enhances imputation accuracy in diverse Chinese populations, addressing critical gaps in non-Han genetic representation. Its inclusion of underrepresented ethnic groups advances equitable genomic research and precision medicine. Future efforts should prioritize ancestral diversity to optimize global applicability.

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P15.014.A Exploring Mosaic NLRP3 Variations: From Chronic Urticaria to Neonatal Autoinflammatory Disease

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Background: NLRP3-associated autoinflammatory diseases (NLRP3-AIDs) encompass a range of inflammatory disorders driven by germline or somatic mosaic variations in the *NLRP3* gene, which cause dysregulated inflammasome activation and systemic inflammation. The clinical manifestations of *NLRP3*-AIDs vary from isolated conditions such as chronic urticaria to more complex, syndromic forms associated with severe systemic inflammation. This study investigates the phenotypic and molecular characteristics of *NLRP3* mosaic variations in patients with systemic autoinflammatory diseases (SAIDs).

Material and Methods: A cohort of 1,000 patients with clinical features ranging from isolated chronic urticaria to syndromic forms of autoinflammatory diseases was evaluated using conventional or high-depth (1,500X) next-generation sequencing (NGS) targeting a panel of SAID genes. Functional assays, including NF-κB pathway activation and ASC speck formation, were performed to assess the gain-of-function (GOF) effects of the identified *NLRP3* variations.

Results: Mosaic *NLRP3* variations were identified in nine patients, with allele frequencies as low as 2–3%, undetectable by conventional NGS. Clinical presentations ranged from isolated late-onset chronic urticaria to neonatal syndromic SAID. Functional studies confirmed a GOF effect in all mutations, activating the NF-κB pathway and/or promoting inflammasome activation (ASC speck formation). Anti-IL-1 therapy led to significant clinical improvement, with earlier initiation preventing severe complications.

Conclusion: This study underscores the importance of high-depth sequencing for detecting low-level mosaic *NLRP3* variations underlying a broad spectrum of chronic inflammatory conditions. Early and accurate diagnosis is crucial for timely anti-IL-1 therapy, significantly improving patient outcomes and preventing complications in NLRP3-AID.

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P15.015.B Multilocus Imprinting Disturbances (MLID) analysis in a cohort of 101 Beckwith-Wiedemann Syndrome patients

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Background: Beckwith-Wiedemann syndrome (BWS) is a genetic overgrowth disorder associated with DNA methylation anomalies in the 11p15.5 region, particularly affecting ICR1 and ICR2. These regions regulate key genes like IGF2 and CDKN1C, essential for growth and development. Multilocus imprinting disturbances (MLID) in BWS, are characterized by aberrant methylation in regions beyond 11p15.5 or other regions, are found in some BWS cases and are relevant for diagnosis and management.

Material and Methods: The methylation status of 101 BWS patients was analyzed by both MS-MLPA and methylation microarrays. All selected patients had previously been diagnosed with BWS due to abnormal methylation in the 11p15.5 chromosomal region and compatible phenotype, using the SALSA MS-MLPA probemix ME030 BWS/RSS kit, while methylation microarrays (Infinium MethylationEPIC v2.0 BeadChip) provided genome-wide CpG site data, identifying additional methylation patterns. Raw data were processed in R and analyzed for differential methylation. Clinical feature differences were evaluated using statistical tests, including Chi-square and Fisher's exact, with significance set at $p < 0.05$.

Results: ICR2 hypomethylation was prevalent in MLID cases (15.84% by MS-MLPA, rising to 26.73% using microarrays). Novel hypomethylated loci (DIRAS3, FAM50B, ZNF331) were detected, expanding the spectrum of epigenetic abnormalities in BWS. Significant loci like GNAS and PLAGL1 were frequently altered, consistent with previous reports.

Conclusion: ICR2 hypomethylation is a diagnostic hallmark of MLID in BWS. Advanced methylation techniques improve diagnostic precision, revealing novel loci. These findings emphasize genome-wide approaches for comprehensive MLID analysis and suggest potential targets for future research and therapy development.

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P15.016.C Charcot-Marie-Tooth disease with *GDAP1* genevariations in a French multicentric cohort

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Background: Charcot-Marie-Tooth disease is the most common inherited peripheral neuropathy, with more than 100 associated genes identified to date. Among these, the *GDAP1* gene is particularly noteworthy due to its dual inheritance patterns—autosomal recessive and autosomal dominant —and its association with both demyelinating (CMT4A or AR-CMTde-*GDAP1*) and axonal forms (CMT2H/AR-CMTax-*GDAP1* and CMT2K/AD-CMTax-*GDAP1*), corresponding to distinct electrophysiological characteristics of the associated neuropathies.

Material and Methods: Our study aimed to analyze a large cohort to better understand the genetic characteristics of *GDAP1*-related Charcot-Marie-Tooth disease. The cohort included 167 individuals, of whom 110 were index cases.

Results: *GDAP1* variants were inherited in an autosomal recessive manner in 58% of cases and an autosomal dominant manner in 42%. In our cohort, we identified 60 distinct variants, among which 25 have never been described in the literature. Consistent with existing literature, the most frequent alterations were p.(Arg120Trp) in dominant cases and p.(Ser193*) in recessive cases. The pathogenicity of the newly identified variants was assessed according to the ACMG guidelines.

Conclusion: This comprehensive analysis of a large *GDAP1* cohort offers valuable insights into the genetic diversity underlying *GDAP1*-associated CMT, enhancing our understanding of this complex disorder.

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P15.017.D Using whole-genome sequencing and related technologies to resolve cases of suspected autosomal recessive rare genetic disease

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Background: In genetic testing for rare diseases, it is a recurring scenario that a single heterozygous pathogenic variant is identified in a gene associated with an autosomal recessive disorder, but no second variant is identified in trans. It is hypothesized that this second variant may be present, however cannot be detected due to the technical limitations of capture-based NGS methods.

Materials and Methods: 17 individuals were identified for whom previous panel testing or exome sequencing identified a heterozygous pathogenic variant in a gene felt to be a good fit for their clinical presentation. All individuals and available parents underwent WGS. Data analysis first prioritized interpretation of variants in the locus of interest. If no second compound variant was identified, a genome wide analysis was performed to identify alternative explanatory variants.

Results: Of the genomes analyzed, WGS identified a second variant in the initial gene of interest for four individuals. An additional four individuals were found to have candidate pathogenic variants in an alternative gene. Additional functional work is currently being undertaken to confirm the pathogenicity of certain variants identified. The remaining 9 are currently unsolved and have been sent for additional investigations including RNAseq and long-read genome sequencing.

Conclusions: These results show the efficacy of short-read WGS to provide a molecular diagnosis in these specific cases of suspected autosomal recessive inheritance. It also highlights the benefit of looking beyond the initial region of interest, especially when an individual's clinical presentation is genetically heterogeneous.

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P15.019.B Investigating rare diseases through multiomic nanopore sequencing approaches

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Rare diseases often require multiple individual assays to understand their aetiology. The additional time, cost, and complexity associated with complex genetic and epigenetic disorders creates a high barrier of entry into research of these systems. Here we explore the technical abilities of nanopore sequencing to address the complexity of rare diseases using a multiomic approach. We analysed 9 cell lines derived from traditional and non-traditional trios of healthy individuals and probands with rare genetic and epigenetic disorders including Rett syndrome, Friedreich's ataxia and alpha-thalassemia. We investigated phased genetic, transcriptomic, and epigenetic variation, as well as higher order structural changes associated with disease state. We performed whole genome sequencing to more than 30x coverage per sample and N50's of 10–20 kb. Using a multiomic approach encompassing phased SNP, SV and methylation calling in combination with chromatin accessibility, chromatin conformation and long read cDNA analysis we demonstrate that skewed X-inactivation and crossing over events are the primary drivers of phenotypic differences between a healthy mother and affected daughter, both with an underlying genetic variant associated with Rett syndrome. In addition we explore how long read sequencing can help resolve challenging medically relevant genes (CMRGs) including characterising the length and composition of repeat expansions, as found in the *FXN* gene, and differentiating

homologous regions of the genome, for example *HBA1/2*. In summary, we present a nanopore-based multiomic approach that leverages information from genetic, epigenetic, transcriptomic and structural components to gain a deeper insight into the mechanisms underpinning complex and difficult to resolve disorders.

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P15.020.C Disordered genome methylation in the Beckwith-Wiedemann syndrome associated with Multi-Locus Imprinting Disturbance

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The monoallelic expression of the imprinted genes is under the control of germline-derived Differentially Methylated Regions (gDMRs). The Beckwith-Wiedemann syndrome (BWS) is an overgrowth and tumor-associated disorder characterized by deregulation of chromosome 11p15.5 imprinted genes. The most frequent molecular defect is loss of methylation (LoM) of the KCNQ1OT1-TSS gDMR (aka IC2). Approximately one-third of the IC2 LoM BWS patients display multi-locus imprinting disturbance (MLID). However, in most of these studies investigation was rather limited and the impact of MLID on the whole genome is unknown. By employing the Infinium Epic methylation array, we investigated the whole epigenome in 65 BWS patients with IC2 LoM. By comparing these patients with 33 control subjects, we identified differentially methylated CpGs within all gDMRs that allowed to separate 30 cases with MLID from 35 cases with only IC2 LoM by hierarchical clustering. By extending the analysis outside of the imprinted loci, we also found that the MLID cohort exhibited higher methylation variation in a consistent fraction of CpGs with respect to the control and non-MLID groups. By whole exome-sequencing, we further demonstrated the presence of maternal-effect pathogenic variants of the Sub-Cortical Maternal Complex genes in 50% of the MLID cases but not in the non-MLID group. In conclusion, these findings support the hypothesis that MLID in BWS is a disorder caused by variants of maternal-effect genes affecting CpG methylation maintenance in the whole genome, including all the imprinted and thousands of further genomic loci, whose impact on the clinical phenotype needs to be evaluated.

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P15.021.D Long-read genome sequencing in pediatric cohort with rare genetic disorders: evaluating diagnostic rates in a Qatari population

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Background: Despite wide adoption of next-generation sequencing in diagnosing rare genetic diseases, over 50% of cases remain unresolved. This diagnostic gap is potentially due to limitations of short-read sequencing (SRS) in resolving complex genome regions. The emerging application of long-read sequencing (LRS) addresses these limitations by generating longer reads (10–500 kb), which enhances detection of structural variants (SVs) and other complex genome regions. This study aims to investigate the utility of LRS in improving diagnostic yield in patients that remained undiagnosed by SRS.

Material and Methods: We performed whole-genome LRS using ONT's PromethION on 68 pediatric patients with genetic diseases at Sidra Medicine. Of these, 48 cases had negative or inconclusive findings from microarray and SRS, while 20 cases served as positive controls with known variants identified using SRS. Sequencing was performed at 20x depth, and data processed with wf-human variation workflow, which includes Sniffles2 for SV calling.

Results: LRS confirmed all variants in the positive cohort (20/20 cases). Additionally, LRS identified four SVs not detected by SRS, leading to an 8.3% increase in diagnostic yield (4/48 cases). These included deletions in TTN and KCNQ2, a duplication in SCNNB1, and a novel de novo inversion in SMARCC2, associated with Autism Spectrum Disorder (ASD).

Conclusion: Our findings highlight the potential of LRS to improve diagnostic yield in cases unresolved by SRS. Future integration of methylation analysis and other complex genomic regions could enhance its utility in rare disease diagnostics.

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P15.022.A An expanded view of uORF variants as causes of mendelian disorders

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Background: Interpretation of variants in upstream ORFs (uORFs) is a significant and complex task in medical genetics. This challenge arises from the limited understanding of how these variants influence downstream CDS translation, especially when variants do not lead to overlap with it.

Material and Methods: We used a previously created uORF_annotator tool to identify and assess the impact of nucleotide variants from HGMD and ClinVar (P/LP/VUS) on uORFs structure and the subsequent change in main CDS translation. Next, we conducted detailed analysis of the identified variants using publicly available databases (GWIPS-viz, TRIPS-viz, gnomAD, CAGE data, etc.).

Results: uORF_annotator revealed 83 variants altering ATG-started uORFs by their elongation or shortening. Among these, 57 variants were found in non-overlapping uORFs, while 26 affected uORFs that initially overlapped with CDS. We classified variants in non-overlapping uORFs based on their potential impact on downstream CDS: (1) uORF elongation resulted in out-of-frame CDS overlapping (most pathogenic variants and eight VUS); (2) four variants elongate uORF in-frame with main CDS, leading to N-terminal protein extension; (3) 45 VUS variants altered uORF length without affecting CDS. These variants may exhibit varying effects on uORF length and distance to the CDS start codon, resulting in diverse impacts on CDS translation. Several groups were also identified among variants in overlapping uORFs. The most interesting included uORF shortening with overlap removal, which may enhance main CDS translation.

Conclusion: Our bioinformatic analysis suggests mechanisms by which uORF variants may influence CDS translation. These hypotheses will be tested in future experimental studies.

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P15.023.B The puzzle of OFD1: one gene, several disorders

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Background: The complex structure of the gene *OFD1* reflects the functional complexity of this pleiotropic protein; involved in biogenesis and maintenance of primary cilia, DNA repair and regulation of protein balance. Four different X-linked ciliopathies have been associated with *OFD1* mutations: OFD syndromes type I, Joubert type 10, Retinitis Pigmentosa 23 and Primary Ciliary Dyskinesia. This evident clinical and genetic heterogeneity suggests different underlying molecular events, that we aim to identify.

Material and Methods: We selected representative variants for the four ciliopathies and generated HK-2 mutant stable cell lines. Their characterization by super-resolution microscopy and OMICs techniques led us to identify specific phenotypes that we are defining with functional assays.

Results: Mutant lines expressing JBS10 and PCD phenotypes exhibit a significant hyper-elongation of the cilium; this suggested a compromised sensorial function of the cilia, which Transcriptomics analyses confirmed. Transcriptomics analyses also revealed up-regulation of genes involved in mitochondrial functions. Indeed, by performing TMRE and mito-stress assays, we found that mitochondria parameters are particularly compromised in the RP23 mutant. This finding is consistent with the emerging correlation between ciliary length and mitochondrial function. In the PCD mutant, we observed the loss of OFD1-interactors with critical roles in actin-mediated movement of cellular substructure, while the OFD1 mutant showed endoplasmic reticulum defects, which we are dissecting by EM analysis.

Conclusion: This experimental approach will shed light on the pathogenetic mechanisms underlying the clinical heterogeneity associated with *OFD1* mutations and could be also applied to other difficult-to-study disease genes in the field of ciliopathies.

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P15.025.D Selective exome screening of newborns: first results of the pilot project

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National Medical Research Center for Obstetrics, Gynecology, and Perinatology named after Academician V.I. Kulakov, Moscow, Russian Federation.

Background: 3.5–5.9% of the population suffers from rare diseases, often manifesting in childhood. Only 10% of genetic disorders have pathognomonic signs in the neonatal period with deterioration in the first year of life, but the genetic disease can be suspected by indirect clinical manifestations and the neonate can be placed in the risk group for selective exome screening (SES).

Material and Methods: Neonatologists and geneticists in 7 perinatal centers selected newborns based on 128 clinical parameters. Whole exome sequencing (WES) was performed on blood samples. Inclusion required ≥2 clinical features; some cases with one feature were analyzed at physicians' discretion.

Results: Of 1306 neonates screened, 174 (13.3%) had causative findings. On average 4 criteria (median) were noted, maximum number of features was noted in 1 child (19), 1 feature - in 47 neonates. The detection rate with 1 feature was 8.5%, dropping to 2.5% if "neonatal screening risk" was excluded. With ≥3 features, the rate was 14.1%. Detection varied by organ systems: 16% for gastrointestinal/neuromuscular issues and lab changes; 22–33% for musculoskeletal, urinary, and facial dysmorphisms. Potentially it makes possible to use these features as the sole for inclusion in the SES. Notably, 41.3% of cases would have been missed without WES. Increasing the number of clinical criteria had minimal impact on detection rates.

Conclusion: SES is effective, achieving a 13.3% detection rate overall. High-yield clinical features could serve as standalone criteria, optimizing inclusion and reducing reliance on multiple indicators.

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P15.026.A Long-read sequencing increases the diagnostic rate for rare diseases

Hannah Titheradge[1], *Lorraine Hartles-Spencer*[2], *Luke Ames*[3], *Jo Stockton*[3], *Derek Lim*[4], *Kai Ren Ong*[1], *Emma Douglas*[1], *Madhura Chandrashekara*[1], *Ataf Sabir*[1], *Deborah Osio*[1], *Mary O'Driscoll*[1],

Denise Williams[1], Helen Brittain[1], Joanna Jarvis[1], Esther Kinning[1], Helen Cox[1], Rebecca Igbokwe[1], Adrian Shields[3], Scott Hackett[5], Abhijit Dixit[6], Claire Searle[6], Andrew Beggs[3]

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Background: We aim to evaluate the utility of Nanopore long-read sequencing (LRS) as a tool to increase the diagnostic rate of suspected monogenic rare disorders for those patients who have no molecular diagnosis despite maximal standard-of-care genomic testing.

Material and Methods: We identified 27 individuals highly likely to have a monogenic disorder, who have no molecular genetic diagnosis, despite reaching the ceiling of investigation as defined by the NHS England within the National Genomic Test Directory. They were recruited as trios and quads (affected patient(s), and their parents). Whole genome sequencing was performed using the Oxford Nanopore Promethion P24 instrument. We have used a range of existing software packages to study participants' genomes for potentially disease-causing variants including, sequence, copy number, methylation variants, and tandem repeats.

Results: We have identified the underlying molecular aetiology in an additional 11% of patients and their families so far including a diagnosis of Coffin Siris syndrome, Familial Adenomatous Polyposis and Roifman syndrome. We will discuss the clinical phenotypes of these patients, the bioinformatic pipeline and downstream analysis tools utilised. We will explore why these variants were not previously identified on genomic testing as well as the characteristics that seem to optimise the diagnostic yield from LRS. Finally, we will consider the feasibility of implementation of Nanopore sequencing into diagnostic testing pathways.

Conclusion: Nanopore LRS has increased the diagnostic rate for patients with likely monogenic disorders in families who have reached the ceiling for investigation in the NHS.

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P15.027.B Long-read genome sequencing for diagnosis of rare diseases

Médéric Jeanne[1], *Mehmet Bugrahan Duz[1], Tina K. Truong[1], Wanqing Shao[1], Bryan Leland[2], Kshitij Amar[3], Nathaniel Edisis[1], Pratiksha Pradhan[1], Priyesh Rughani[2], Rebecca Stubbs[2], Kenney Tuyen[2], Talia Tzadikario[3], Carly Tyer[2], Scott Hickey[2], Sissel Juul[2], Shira Rockowitz[1], Piotr Sliz[1], Catherine A. Brownstein[1], Miten Jain[3], Alan H. Beggs[1], Wendy K. Chung[1]*

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Background: Overcoming the limitations of traditional short-read sequencing, long-read genome sequencing (LRGS) allows variant phasing, methylation analysis, detection of structural variations (SVs), copy number variations (CNVs), and short tandem repeat expansions, while maintaining accurate single nucleotide variants (SNV) and small indel detection. To explore the utility of LRGS, we conducted nanopore sequencing in a cohort of undiagnosed patients.

Material and Methods: We selected probands with rare diseases who consented to research-based genomic analysis. DNA was sequenced on Oxford Nanopore Technologies' PromethION instruments. The EPI2ME-wf-human-variation workflow was employed for variant calling, methylation analysis and phasing.

Results: The BEACON Project aims to apply LRGS sequencing to 500+ samples. Here, we assessed 52 participants (33 affected individuals), including 21 singletons, 1 duo, and 10 trio cases with congenital diaphragmatic hernia (6), esophageal atresia (21), and other rare conditions (6). LRGS informed the diagnosis in nine individuals (28%), including two with congenital diaphragmatic hernia, six with esophageal atresia, and one with another rare condition. Findings included three CNVs and six SNVs or indels. In an overgrowth case, LGRS identified a 6 kb 3'UTR deletion of *HMGA2*, a known overgrowth gene, and methylation analysis was suggestive of overexpression, supporting its pathogenicity. Additionally, in a case of esophageal atresia, LRGS identified a truncating deletion in *ZZZ3*, a candidate gene involved in chromatin remodeling.

Conclusion: LRGS is an effective tool for identifying a wide range of variants including SVs, CNVs, methylation abnormalities, as well as SNVs and indels with the potential to be an effective diagnostic tool.

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P15.029.D Population screening for rare genetic diseases in Quebec, Canada: a proof of concept benefiting individuals from Saguenay-Lac-Saint-Jean, Charlevoix and Haute-Cote-Nord

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Background: Few autosomal recessive genetic disorders are more frequent in the Saguenay-Lac-Saint-Jean (SLSJ), Charlevoix (Cx), and Haute-Côte-Nord (HCN), three regions of the Province of Quebec, Canada, well known for their shared founder effect. Since 2010, a provincial carrier screening program covers the four most common disorders, with carrier rates between 1/19 and 1/23. Recently published clinical data suggested that additional disorders should be considered in the carrier screening program[1]. This study aimed to identify missing diseases and pathogenic variants eligible for inclusion in the carrier screening program.

Material and Methods: We used clinical data and variants identified in a recent unbiased study[2] to identify pathogenic variants causal of recessive disorders presenting as more frequent in the SLSJ, Cx and HCN populations. Their carrier frequencies were confirmed in an independent and randomly selected cohort of 1000 reporting at least one grandparent from the SLSJ, Cx, or HCN regions using specific TaqMan real-time PCR Assays. A carrier rate threshold of 1/200 to assess potential eligibility for carrier screening was determined taking in consideration the recommendations of multiple regulatory bodies.

Results: A total of 23 disorders and 31 variants were identified and selected for carrier rate assessment and confirmation. Among them, 23 variants (21 disorders) reached a carrier frequency of 1/200 or higher.

Conclusion: We have identified 23 novel pathogenic variants with a carrier rate of 1/200 or higher. These are causal of very severe recessive diseases for which no curative treatment is available. They should be considered for carrier screening program.

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P15.030.A Genetic newborn screening by TREAT panel targeted sequencing in a large European cohort: pipeline design and first output results from the Screen4Care EU project

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The EU Screen4Care initiative has developed a 245-gene panel of treatable disorders for genetic newborn screening (gNBS). This study outlines the data flow and analysis strategy, from patient recruitment to research report. The TREAT panel, designed using Roche technology, is supported by GenomeUP software for metadata collection and an Illumina BaseSpace account for secure raw data transfer. Recruitment materials, including multilingual videos and flyers, were distributed at participating maternity centers from Italy, Germany, and France. Samples are collected on custom dried blood spot cards developed for the project. The cards are periodically shipped to the sequencing center for panel sequencing. Validation of the panel using 24 positive control cases addressed complex variants, including short deletions, large deletions, duplications, and variants in pseudogenes (e.g. SMN1). Gene coverage assessments identified challenges in reporting certain genes, such as CBS. Results showed high accuracy and a very low false positive rate. Based on those, a standard procedure was established for automated filtering. This includes identifying (likely) pathogenic variants based on ACMG criteria and integrated databases, including ClinVar. Filtering parameters were tailored to each gene's inheritance pattern and specific variant types. Protocols involving European Reference Networks have been set up to evaluate variants of uncertain significance. The initial results from validation and first enrolled samples highlight challenges in implementing gNBS at scale. Over the coming months, the workflow will expand to screen up to 18,000 newborns, improving our understanding of gNBS, early diagnosis and treatment of rare genetic disorders.

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P15.031.B Identifying molecular diagnoses using an RNA-guided workflow to interpret variants of unknown significance and candidate genes with gene network analysis to accelerate rare disease diagnostics

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Current genome diagnostics provides a molecular diagnosis for 20–80% of patients. Interpreting variants remains a challenge, as WGS delivers 4 to 5 million variants per individual, most of them non-coding, which can be of unknown significance or are not linked to a specific gene.Within the long-loving MOLGENIS project, the variant interpretation pipeline (VIP) is widely used to annotate variants with best-in-practice algorithms and protocols from routine diagnostics, supporting the usage of ACMG guidelines. Newly emerging methods like sequence-based models are able to prioritise variants with likely functional consequences, while gene outlier analysis can identify genes that are aberrantly expressed. Combining these new tools with standardised workflows, like VIP, could increase diagnostic yield.Here, with the expertise of clinical geneticists, clinical genetic laboratory specialists and bioinformaticians, we combined VIP, OUTRIDER and FRASER into a single simplified workflow to pinpoint variants causing deregulated gene expression and splicing in whole blood (WB) and fibroblasts (FB). Additionally, Borzoi and Pangolin are used, zooming in deeper on expression and splicing altering variants and GADO to identify new candidate genes. This workflow provides stand-alone interactive reports for immediate clinical interpretation on a patient level.We studied WB and FB RNA sequencing data, germline WGS and HPO data for 300 undiagnosed cases from the the American Undiagnosed Disease Network (UDN) with clinical diagnoses ranging from rare neurological and neurodevelopmental to haematological diseases. By leveraging the new toolings together with expertise from clinical geneticists, we identified 8 strong candidate variants explaining the clinical phenotype of the patient.

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P15.033.D Investigating the genotype-phenotype relationshipfor TSC1 and TSC2pathogenic variants in a population cohort; implications for genomic newborn screening

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Background: Despite the inclusion of tuberous sclerosis genes *TSC1* and *TSC2* in genomic newborn screening programs worldwide the penetrance of pathogenic variants in these genes in general populations remains unknown.

Materials & Methods: We analysed genome sequencing data on 500,000 UK Biobank participants, identifying individuals with high confidence, loss-of-function or previously reported pathogenic variants (ClinVar 2-star or above) in *TSC1* and *TSC2*. These were appraised according to ACMG criteria retaining likely pathogenic and pathogenic variants. In individuals with these variants phenotypic data, including ICD10, ICD-9, self-reported disease and primary care read codes, were interrogated for symptoms indicative of Tuberous Sclerosis.

Results: 13 *TSC1* and 9 *TSC2* variants were identified, in 13 and 12 participants, respectively. Only 5 had a diagnosis of tuberous sclerosis (4 *TSC1*, 1 *TSC2*), whilst a further 4 showed at least one feature of tuberous sclerosis. Notably, 16/25 (64%) individuals identified, all aged ≥55 years, had no recorded features of tuberous sclerosis. Despite analysis of all rare coding / splice variants in TSC1 and TSC2, 10/18 individuals self-reporting as having Tuberous Sclerosis had no explanatory variant.

Conclusion: Our findings challenge the previously reported near-complete penetrance of tuberous sclerosis, revealing a substantial number of asymptomatic variant carriers are present in the general population. While penetrance may be affected by healthy cohort bias, the existence of these individuals has important implications for newborn screening programs, clinical counselling and variant interpretation. Additionally, our finding of errors in clinical and self-reported diagnosis has wider implications to similar studies.

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P15.034.A Applications of the new tool: VUS Notifier

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Background: More than 300 million people worldwide are affected by a genetic rare disease (RD). Currently, comprehensive genetic testing, including whole-exome and whole-genome sequencing, has become the first-tier approach for determining the molecular genetic basis of these conditions. However, many genetic variants identified through these tests lack sufficient data for definitive classification as either pathogenic or benign and are therefore designated as variants of uncertain significance (VUS). Over time, as new scientific evidence emerges, these VUS may be reclassified as either pathogenic or benign.

Material and Methods: VUS Notifier tool facilitates the search for specific genetic variants by integrating data from four publicly available databases: ClinVar, VEP, LOVD and PubMed. More importantly, users can save variants of interest, and the system provides notifications when new information about these variants becomes available in the databases or scientific literature. Tool was developed using Python for backend and Next.js for frontend.

Results: Novel freely available tool, VUS Notifier, was developed and it is available at https://www.vusnotifier.app/

Conclusion: The VUS Notifier addresses the challenge of delivering timely updates on VUS and other genetic variants, offering a range of applications. First, it autonomously searches for new information, eliminating the need for manual monitoring by patients, geneticists, or clinicians. Additionally, it can facilitate the re-analysis of unresolved RD cases. Furthermore, the tool could be used to systematically collect published data, aiding in the selection of the most attractive genetic targets for pharmacochaperones. Notably, this is the first tool to integrate multiple resources while providing automated notifications.

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P15.035.B Polyadenylation-Disrupting Variants in Rare Disease: A Genome-Wide Analysis and Experimental Validation

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Background: Cleavage and polyadenylation of mRNAs is essential for transcription termination and gene expression, yet variants disrupting conserved sequences essential to this process are often overlooked as putative causal variants in individuals with rare Mendelian disorders.

Material and Methods: Here, starting with variant effect predictions from advanced machine learning models, we conducted a systematic genome-wide analysis of germline variants inferred to impact cleavage and polyadenylation in mRNA 3'-untranslated regions (3'UTRs) in individuals with rare diseases in the Genomics England 100,000 Genomes Project.

Results: We identified enrichment of ultrarare alleles predicted to disrupt polyadenylation in a large cohort of undiagnosed probands with rare disease disorders from the Genomics England 100k dataset. These alleles are predominantly located in the 3' UTRs of genes where protein-coding variants have previously been identified as causal in rare diseases. Experimental validation of a putative causal variant predicted to disrupt polyadenylation of SLC16A2 in a proband with intellectual disability, confirmed the allele's deleterious impact on gene expression.

Conclusion: These results demonstrate that variants affecting cleavage and polyadenylation can have functional consequences and should be considered in the genetic diagnosis of rare disorders.

C.O.I. Disclosure:

Y. Kainov: None. **C. Dias:** None. **T. Hubbard:** None.

P15.036.C Genotype-Phenotype Correlations of Rare SCHEMA Variants: Insights into Cognitive, Demographic, and Neuroimaging Features from the UK Biobank

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Background: Genetic studies have established the role of rare variants in schizophrenia aetiology. The Schizophrenia Exome Sequencing Meta-Analysis (SCHEMA) consortium identified 32 genes significantly associated with schizophrenia risk. This project examines rare variant carriers of SCHEMA genes in the UK Biobank and compares their phenotypic traits with non-carriers.

Material and Methods: Genotypic analyses were conducted using the UK Biobank Research Analysis Platform. Variant carriers that passed quality control were extracted from population-level exome OQFE data in PLINK format, with an allele frequency threshold of <0.000005.Phenotypic comparisons were performed across three groups: (1) carriers of the top ten SCHEMA genes ($n = 652$), (2) carriers of genes ranked 11 to 32 ($n = 1506$), and (3) all SCHEMA gene carriers ($n = 2074$) versus the rest of the UK Biobank population.

Results: Across all groups, rare variant carriers exhibited significantly greater socioeconomic deprivation ($p = 2.87e-04$, $p = 5.622e-07$, $p = 1.566e-10$), lower fluid intelligence scores ($p = 0.001336$, $p = 2.91e-08$, $p = 1.308e-09$), and poorer performance on cognitive tasks, including prospective memory ($p = 0.0001215$, $p = 0.003548$, $p = 8.743e-06$). Carriers in groups 1 and 3 took longer to correctly identify matches ($p = 0.001701$, $p = 0.001698$), while groups 2 and 3 showed a higher likelihood of perceiving unreal visual stimuli ($p = 0.04121$, $p = 0.03141$). Imaging analyses revealed significantly smaller ventricular cerebrospinal fluid volumes in carriers ($p = 0.04376$), normalised for head size. However, no significant associations were found with ICD-10 mental and behavioural disorders or schizophrenia related diagnoses.

Conclusion: While rare SCHEMA variant carriers show disadvantages in cognitive and demographic factors, these do not translate into a significantly increased risk of psychiatric disorders.

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X. Chen: None. **A. McQuillin:** A. Employment (full or part-time); Significant; UCL. **N. Bass:** A. Employment (full or part-time); Significant; UCL.

P15.037.D Answering the unmet need of rare disease diagnosis through high throughput functional genomics

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Background: A major challenge in diagnostics is that true causative variants often require functional evidence to be reclassified from variants of uncertain significance (VUS). Clinically validated, accessible functional methods are needed to fulfill this purpose. Untargeted mass spectrometry (MS)-based proteomics has gained attention as a functional genomic technology due to its increasing ability to detect changes in thousands of proteins in common clinical sample types and its high-throughput nature.

Material and Methods: A retrospective study was conducted on lymphoblast or fibroblast cells from 23 randomly selected rare disease patients with their diagnosis blinded to investigate the utility of MS-based proteomics. Relevant HPO terms and 1–2 candidate genes were provided for each case. The untargeted proteomics results were reviewed with respect to candidate proteins based on 1) abundance, 2) protein complex association, and 3) interacting or binding partners noted in published literature.

Results: We observed a similar number of recessive and dominant cases, de novo, homozygous, and compound heterozygous variants and a range of variant types (missense, frameshift, splicing) in our cohort. Nearly 50% of the cases showed a significant reduction in protein abundance, providing direct evidence of likely pathogenicity for the candidate variants. The candidate proteins exhibited an 80% mean reduction in recessive conditions compared to 20% in dominant conditions. Protein complex and interaction analyses were explored for the remaining cases.

Conclusion: Proteomic analyses will likely provide evidence to support variant pathogenicity in a high proportion of monogenic disorders and across all inheritance patterns and for all common variant types.

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P15.038.A Beyond *CFTR*: Unraveling the Genetic Basis of Elevated Neonatal IRT in Non-CF Newborns

Francesca Rosamilia[1], *Ummara Sarfraz*[1,2], *Giovanni Fiorito*[1], *Carola Di Meo*[1], *Maria Baffico*[3], *Giulia Camisassi*[4], *Michela Cassanello*[5], *Elvira Sondo*[5], *Vito Terlizzi*[6], *Laura Barrocu*[7], *Dorota Sands*[8,9], *Katarzyna Zybert*[8,9], *Mohamad Maghnie*[5], *Isabella Ceccherini*[10], *Paolo Uva*[1], *Carlo Castellani*[4]

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Background: Cystic Fibrosis (CF) is the most common severe autosomal recessive disease in Europe, affecting the respiratory tract and exocrine pancreas, also involving altered lipid metabolism. Measurement of the pancreatic enzyme immunoreactive-trypsinogen (IRT) is the first tier of screening even if it implies the identification of False Positives (FP), i.e. non-CF infants with elevated IRT. However, the reasons behind the raised >IRT levels in FP newborns remain unclear and could suggest a genetic contribution.

Material and Methods: Whole Exome Sequencing (WES) was performed on three groups of screened newborns: true positives (TP i.e.CF-confirmed), true negatives (TN, i.e. CF-negative with normal IRT), and FP. Two complementary statistical approaches have been applied: a Bayesian method for rare variants and a logistic regression model for common variants.

Results: WES from 293 CF Newborn samples were investigated: in detail 40 TP, 103 FP, 119 TN and 31 SPID (Borderline Cases). The combination of the Bayesian and logistic approach enables to highlight the contribution of both common and rare variants in such a complex phenotype. The comparison between FP and TN, using both methods, suggested a polygenic risk model, where multiple markers contribute to elevated IRT phenotype. *CFTR* was causative only in TP, while additional genetic factors may underlie elevated IRT in FP.

Conclusion: The present findings improve non-CF detection and enhance NBS positive predictive value. Identifying risk alleles could refine the management of uncertain cases, enabling personalized follow-up. Validation of the results is ongoing and will be presented at the meeting.

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P15.039.B Genomic analysis in hereditary neuropathy: insights from a nationwide French cohort

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Background: Hereditary neuropathies pose a significant diagnostic challenge due to their clinical and genetic heterogeneity. Thanks to the national program Plan France Médecine Génomique 2025, whole genome sequencing (WGS) is now available as part of clinical care in France. This study evaluates the impact of WGS on the diagnosis of hereditary neuropathies before the age of 50.

Material and Methods: From February 2023 to December 2025, 186 WGS requests were received for patients with suspected hereditary neuropathies after prior gene panel testing. 97 cases underwent the Auragen pipeline and 89 SeqOIA. Of these, 109 analyses were completed, 26 are ongoing and 51 are pending.

Results: Of the 109 cases processed, 35 (32%) yielded a definitive genetic diagnosis, identifying pathogenic variants in known neuromuscular genes. Six cases (6%) revealed variants of uncertain significance requiring further validation. Sixty-eight cases (62%) were inconclusive, reflecting the genetic complexity of inherited neuropathies. WGS identified several new candidate genes, and in some cases, this analysis led us to revise the initial diagnosis and suggest appropriate management.

Conclusion: This study highlights the role of WGS in reducing the number of undiagnosed patients with inherited neuropathies. A molecular diagnosis was established in 32% of cases, a high proportion given that all had previously undergone gene panels, thus reducing the number of patients in a diagnostic odyssey. In addition, WGS has proved invaluable in identifying novel disease-associated genes. In conclusion, our nationwide French experience strongly supports WGS as a diagnostic tool for hereditary neuropathies, especially for young patients.

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P15.040.C Using zebrafish model to investigate complex hereditary spastic paraplegia caused by EPT1 variants

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Background: Hereditary Spastic Paraplegia (HSP) comprises a broad and heterogeneous group of inherited neurodegenerative disorders. Among the identified spastic paraplegia genes, 19 are involved in membrane phospholipid metabolism. The Kennedy

pathway is a major biosynthetic route for phosphatidylethanolamine (PE), in which *EPT1* encodes ethanolaminephosphotransferase converting CDP-ethanolamine to PE. Biallelic loss-of-function mutations in *EPT1* have been associated with SPG81.

Material and Methods: To investigate the role of ept1 in HSP, we generated ept1 exon 5 (ex5) knock-out mutants and crispants using the CRISPR-Cas9 system. Phenotypic characterization, RNA sequencing, and lipidomic profiling were performed in these zebrafish models.

Results: The *ept1* ex5 knock-out mutants harbored an 8-bp deletion in the catalytic domain and were viable, exhibiting a 60% reduction in *ept1* mRNA levels. Compared to wildtypes, homozygous knockouts displayed significant impairments in locomotion, reduced body length and head width. Transcriptomic analysis revealed downregulation of genes involved in light response, synaptic activity, and lipid metabolism, whereas genes associated with immune signaling and neuroinflammation were upregulated. Lipidomic profiling showed a reduction in total PE and PC levels in knockouts, with plasmenyl-PE being most severely affected. Through crispant analysis, we found that both *ept1* and *cept1b* crispants exhibited significantly impaired locomotion; however, only *ept1* crispants displayed reductions in body length and head width.

Conclusion: The *ept1* knock-out mutants and crispants recapitulate movement disorder and developmental delay observed in SPG81 patients, making them valuable models for disease research. Moreover, our findings suggest that neuroinflammation may play a crucial role in HSP pathogenesis and could serve as a potential therapeutic target.

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P15.041.D Identification and genotyping of pathogenic short tandem repeat expansions using long-read sequencing

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Background: Movement disorders such as ataxia and myotonic dystrophy are frequently caused by short tandem repeat (STR) expansions. These expansions can extend up to several kilobases in length and exhibit complex motif variations, making them difficult to detect using state-of-the-art approaches.

Material and Methods: Nanopore long-read whole genome sequencing (lrWGS) was performed on positive controls with prior known pathogenic repeat expansions in the genes *ATN1, ATXN1, ATXN2, ATXN8OS, CNBP, C9orf72, DAB1, DMPK, FGF14, FMR1, FXN, HTT, NOP56* and *RFC1*. Repeat loci were evaluated and manually genotyped using IGV (Integrated Genome Viewer) as well as automatically genotyped with tools such as Straglr. Subsequently, lrWGS was applied to a cohort of unsolved ataxia cases.

Results: Even long and complex repeat expansions in the available positive controls could be accurately analyzed manually using lrWGS. However, exact genotyping was complicated by variable motifs and repeat sizes as for some STRs like in *NOP56* a high somatic variability occurs. Hitherto, this also impedes the automatic genotyping of *ATN1, CNBP, DAB1, FGF14* and *NOP56* by Straglr. So far, seven patients from our cohort were subjected to lrWGS and two of them were found to have a clinically relevant repeat expansion (*ATXN8OS* and *FGF14*).

Conclusion: Nanopore lrWGS is a suitable method for genotyping various disease-associated STRs. However, somatic variability remains a challenge that can be addressed by increasing the sequencing coverage.

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P15.042.A Human sex determination and associated pathologies as a model to understand developmental gene regulation

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Background: Our analysis of patients with sex-reversal has identified many genes involved in mammalian sex-determination (SD). Despite extensive exome studies >50% of cases remain idiopathic. We hypothesize that unexplained cases are due to variants in gene regulatory elements (GREs) that may define gene regulatory networks (GRNs). Recently, we defined a novel *SRY* enhancer and identified SNVs in it, associated with 46,XY sex-reversal (PMID: 38555298). We have developed a multi-omic genome-wide approach to identify functional GREs in human SD and predict GRNs.

Material and Methods: Single-cell RNAseq and ATACseq data from human fetal gonad atlas (PMID: 35794482) was reanalysed by Scenic+, to define candidate GREs (cGREs). Transcription factor (TF) binding-site enrichment and mutational constraint in cGREs were determined using pycistarget and Gnocchi respectively. We defined cGREs corresponding to human accelerated regions (HARs, PMID: 27667684). Rare/novel variants from genome sequences of >160 individuals with unexplained sex-reversal were mapped to cGREs and functional variants prioritized using CADD, FinSurf and Fabian.

Results: We defined 18,932 and 18,200 cGREs in Sertoli and pre-granulosa cells with a mean Gnocchi score of 7.90 and 7.88 respectively. Using our pipeline, we observed an enrichment of binding-sites for known SD TFs in cGREs: e.g: SOX9 (Normalized Enrichment Score (NES) = 12.95), NR5A1 (NES = 10.98) in Sertoli and LHX9 (NES = 5.66) in pre-granulosa cells. As proof of principle, we identified a previously reported *SOX9* GRE (eSR-A (PMID: 30552336)).

Conclusion: Our approach dissects the role of GREs and GRNs in SD providing a unique model to further understand developmental gene regulation.

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P15.043.B Investigating the interplay between prematurity and genetic variation in the context of developmental disorders

***Patrick Campbell**[1], Olivia Wootton[2], Sarah Richardson[3], Sarah J. Lindsay[2], Qin Qin Huang[2], Erwan Delage[2], Sana Amanat[2], Michael A. Simpson[1], Elizabeth J. Radford[2], Hilary C. Martin[2]*

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Background: Rare damaging genetic variation accounts for a large proportion of the risk of rare developmental disorders, but common genetic variants, as well as environmental factors such as prematurity, also contribute. Little is known about the interplay between genetic factors and preterm birth in rare developmental disorders, particularly in influencing phenotypic outcomes.

Methods: We use phenotypic and genetic data from 21,712 patients with developmental disorders recruited for clinical sequencing, 16% of whom were born prematurely.

Results: In line with past work, we show that common variants associated with higher educational attainment are associated with longer gestational duration. We advance existing knowledge by demonstrating that this association is likely to be the result of genetically-influenced parental traits or confounders, rather than direct genetic effects, and that the presence of a monogenic diagnosis modifies this association. We identify genes and gene sets enriched for diagnostic mutations amongst preterm probands. In our sample, pathogenic de novo mutations account for a greater proportion of developmental disorder cases among term probands compared to preterm probands, consistent with a liability-threshold model of aetiology. However, we find an excess of likely damaging de novo variants in genes that are not currently associated with developmental disorders among preterm and term undiagnosed probands. In a phenotype analysis, we find that prematurity is associated with more severe clinical presentations and that prematurity and monogenic diagnoses contribute independently and additively to severity.

Conclusions: Our findings emphasise the importance of considering prematurity in understanding and predicting outcomes in rare developmental disorders.

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P15.044.C Evaluating the Utility of Face2Gene in 290 Molecularly Diagnosed Cases: Insights from the Telethon Undiagnosed Disease Project

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Background: Face2Gene (FDNA, Inc.), an AI-driven tool designed to analyze facial features for syndrome identification, incorporates two main components: DeepGestalt, trained on 300 rare genetic syndromes, and GestaltMatcher, developed for the identification of ultra-rare and novel syndromes. DeepGestalt can be combined with Human Phenotype Ontology (HPO) analysis to enhance diagnostic accuracy.This study evaluates the utility of Face2Gene's tools in a cohort of 290 molecularly diagnosed cases from the Telethon Undiagnosed Disease Project.

Material and Methods: Frontal facial photographs of patients with confirmed molecular diagnoses were analyzed. Predictive performance was assessed by comparing the software's top-30 ranked diagnoses against the known molecular diagnoses.

Results: DeepGestalt correctly identified the molecular diagnosis within the top-30 predictions for 83 cases (including 51 cases in the top 3). Incorporating HPO terms alongside image-based predictions removed 9 patients from and added 7 to the top-30 list, slightly reducing overall performance while expanding the range of potential diagnoses (n = 90). Predictions based solely on GestaltMatcher confirmed 13 diagnoses, with minimal overlap (n = 3) between the two algorithms.

Conclusion: In our cohort, the largest from an Undiagnosed Disease Project study, the three tools collectively identified diagnoses in 100 out of 290 patients (34%), with the majority relying on facial feature-based predictions.

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P15.045.D Multi-omic analyses in rare disease diagnosis for individuals who remain undiagnosed after clinical genomic sequencing

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Background: Genomic technologies are revolutionising rare disease diagnosis and enabling precision child health. However, there remain significant gaps in our ability to interpret genomic findings and understand their biological impact. Rare Diseases Now (RDNow) is a multifaceted, clinically integrated rare disease program utilising multi-omic analyses to boost diagnostic yield.

Material and Methods: Individuals who remain undiagnosed after clinical exome/genome sequencing are enrolled on referral from genetics or other medical subspecialties. A personalised testing strategy is designed from a suite of technologies including transcriptomics, proteomics, deep sequencing, long-read sequencing or other functional analyses depending on clinical phenotype and genomic findings.

Results: Of the 392 families recruited, sequencing and analysis is complete for 170 families and for 92 of them we have identified the genetic cause for their condition (54% diagnostic yield). Multi-omics was necessary in 16/92 (17.4%) of diagnosed cases and required comprehensive interdisciplinary collaboration. Other approaches included data reanalysis, deep sequencing, and global matchmaking and functional studies for novel gene discoveries.- Transcriptomics and proteomics were especially useful in refuting pathogenicity of variants of uncertain significance.

Conclusion: We demonstrate that a personalised multi-omic approach informed by clinical deep phenotyping is increasingly important for the diagnosis of patients with undiagnosed rare diseases. Understanding the optimal deployment of these complementary technologies and scalability remain ongoing priorities to deliver integrated multi-omics for rare disease diagnostics.

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P15.046.A Genetic and immunological features in patients with MHC class II deficiency

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Background: Major histocompatibility complex (MHC) class II molecules are master key in antigen presenting process during CD4+ lymphocytes priming. MHC class II genes transcription is regulated by four transcription factors: CIITA, RFXANK, RFX5 and RFXAP. Defects in these factors result in major histocompatibility complex class II expression deficiency, a primary combined immunodeficiency frequent in North Africa. Autosomal recessive mutations in the RFXANK gene have been reported as being the principal defect found in North African patients with this disorder.

Material and Methods: Here, we describe genetic, immunological and clinical features of 11 unrelated Algerian patients whose monocytes display a total absence of MHC class II molecules. They shared mainly the same clinical picture which included protracted diarrhoea and respiratory tract recurrent infections.

Results: Genetic analysis revealed that 9 of the 11 patients had the same RFXANK founder mutation, a 26 bp deletion (named I5E6-25_I5E6+1, also known as 752delG26).

Conclusion: Genetic and Immunological findings in our series may facilitate genetic counselling implementation for Algerian consanguineous families. Further studies need to be conducted to determine 752delG26 heterozygous mutation frequency in Algerian population.

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P15.047.B The CILCAD study: clinical and genetic characterization of a multi-generational p.Arg1231Cys mutation carrying pedigree from a Cilento founder population

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Background/Objectives: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL, OMIM 125310) is an adult-onset, inherited small vessel disease caused by NOTCH3 mutations and characterized by a high phenotype variability. We have studied a large and multigenerational sample of individuals (connected in a 17-generation pedigree) from a founder population of South Italy carrying the widespread p.Arg1231Cys mutation to assess the phenotype variability in this sample and to identify additional genetic factors that could modulate CADASIL phenotype.

Methods: Clinical data were compared between p.Arg1231Cys mutation carriers (CILCAD) and the NON-Carriers from the same population; also, MRI features in CILCAD were compared to those detected in UK-population samples. NOTCH3-region haplotypes were analyzed to define the minimum haplotype shared by the CILCAD and identify novel genetic contributions to the disease phenotype.

Results: We found an increase in neurological manifestations in the CILCAD compared to NON-Carriers from the same population; however, our data suggest a milder disease phenotype associated with the p.Arg1231Cys mutation in the CILCAD. Moreover, we found that CILCAD subjects have lower total cholesterol and LDL levels than the rest of the population. Indeed, NOTCH3-region haplotype sharing analysis revealed that the haplotype carrying the p.Arg1231Cys mutation in CILCAD also carries lowering cholesterol alleles.

Conclusions: We hypothesize that additional genetic and/or environmental factors could contribute to a milder phenotype observed in CILCAD. We also believe that this sample could represent a useful resource for identifying novel genetic and environmental factors implicated in the disease, contributing to an improved understanding of CADASIL pathogenesis.

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P15.048.C Whole-exome Sequencing Reveals Mutational Profiles Associated with Medulloblastoma

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Background: Relapse is the leading cause of death in patients with medulloblastoma (MB), the most common malignant pediatric brain tumor. Understanding the mechanisms underlying recurrence could lead to more effective therapies targeting tumor relapses.

Material and Methods: To investigate the mutational differences between primary and recurrent MB across four molecular subtypes, we recruited 28 patients with primary MB who experienced tumor recurrence, collecting paired samples from a total of 56 cases for whole-exome sequencing (WES). We analyzed differential mutational events at the levels of variants, copy number variations (CNV), genes, and SNP loci for each subtype between primary and recurrent tumors.

Results: In the G3 tumor, recurrence-specific alterations were observed in the driver genes *CTNNB1* and *IDH1*. Different CNV segments showed amplification or deletion across the subtypes, and Genome-Wide Association Studies (GWAS) revealed significant SNP loci in the G3 and G4 subtypes. Recurrent tumors exhibited a higher mutational load compared to primary tumors.

Conclusion: This study enhances the understanding of the mutational differences between primary and recurrent MB across different molecular subtypes. Identifying these molecular alterations can aid in developing more effective therapeutic strategies for targeting tumor relapses in MB.

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P15.049.D Genomic variants from whole-genome sequencing of 100 families with neurodevelopmental disorders in Valle d'Aosta, Italy

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Groppo[1], Vittoria Perseghin[1], Martina Toscano[1], Erika Bibbò[1], Serena Donetti Dontin[3], Cecilia Cargnelutti[3], Mèlody Colliard[3], Alessandro Rosina[3], Anna Maria Beoni[3], Cristina Bèrard[3], Alessandro Coppe[2], Paolo Serravalle[4], Fabio Landuzzi[2], Francesco Musacchia[1,5], Amoroso Antonio[1,6], Remo Sanges[5,7], Andrea Cavalli[2,8,9], Manuela Vecchi, PhD[1], Laure Obino[3], Stefano Gustincich[1,5]

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Background: Neurodevelopmental disorders (NDDs) are complex conditions with a strong yet not completely understood genetic component. NDDs genetic architecture in under-represented communities is poorly studied. We present a comprehensive whole-genome sequencing (WGS) analysis to investigate the genetic basis of NDDs in 298 individuals from 100 families resident in Valle d'Aosta, Italy. This study is part of the "5000genomes@VdA" project, which aims to characterize the genomic landscape of the healthy population and residents affected by various conditions.

Material and Methods: We used a comprehensive computational pipeline, rigorous filters and extensive manual curation to detect both de novo and inherited short variants (SNVs and indels) as well as several classes of structural variants.

Results: We identified likely pathogenic variants in 26 out of 110 of our probands, for a diagnostic rate of 23.6%. Additionally, we detected 44 VUS potentially contributig to the probands phenotypes. Notably, four likely gene-damaging variants affected genes not previously robustly implicated in specific NDDs: *KALRN, BAZ2B, ALDH18A1, SYTL4* and *COL6A1*. Additionally, the examination of rare inherited and de novo copy number variants (CNVs) suggested a role for *PRDM16, DOCK8, NPAS1, NECTIN3*, and *GLRA1;* as well as multiple CNVs located in the 15q13.2-q13.3 and 15q11.2 *loci*. These variants may primarily function as susceptibility factors with incomplete penetrance.

Conclusion: This comprehensive approach highlights the utility of WGS in uncovering complex genetic factors and suggests new candidate genes and variants for further investigation.

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P15.050.A Automated Uniparental Disomy Detection: Insights from a Large Rare Disease Cohort

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Uniparental disomy (UPD) is a copy-neutral genetic anomaly where both copies of a chromosomal pair, or segments, originate from a single parent. This alteration can have significant clinical consequences, yet detecting UPD is challenging due to its rarity and limited understanding of its phenotypic presentation. We report a large UPD cohort analysis after integrating automated UPD detection methods into our routine rare disease diagnostic DNA sequencing pipeline, using solo/trio Exome- and solo Genome Sequencing. Solo UPD analyses utilized regions of homozygosity (ROH), while trio UPD detection employed Mendelian inheritance error principles with Bayesian machine learning methods. Among 88,624 analyzed samples, 47 previously undiagnosed potential UPD cases were identified and CNVs excluded. Of these, 37 were confirmed as true UPD cases by either MLPA or CMA, four were reported as ROH regions (chr 1, 2, 21), and six were considered potential UPD without available confirmatory methods (chr 1, 2, 6, 9, 21). Additionally, 34 UPDs associated with established imprinting disorders[1], including chromosomes 6 (one patient), 7 (six Silver-Russell Syndrome patients), 14 (one Kagami-Ogata Syndrome and two Temple Syndrome patients), 15 (13 Angelman Syndrome and eight Prader-Willi Syndrome patients), and 20 (three GNAS disorders). Of the 47 suggestive UPDs, trio analyses detected five isodisomies and one heterodisomy. Among solo samples were 29 likely isodisomies, based on confirmatory results, and 12 ambiguous calls. Our study demonstrates the reliable application of automated UPD detection in a diverse patient cohort, enhancing diagnostic accuracy and understanding of UPDs, including potential novel and previously underexplored associations.

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P15.051.B Using GPT-4 chatbot for genomic data interpretation in monogenic disorders

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Background: Artificial intelligence technologies are increasingly being used in medicine. We investigated the use of GPT-4, a generative large language model-based chatbot, in exome sequencing variant prioritization.

Material and Methods: For each case, the exome analysis platform (Emedgene variant interpretation software; Emedgene Technologies, an Illumina company) suggests a list of approximately 10 "most likely" (ML) variants to solve the case. We retrospectively applied GPT-4 in 43 solved exome sequencing cases. For each case, we used the ML variant list that included the

true diagnosis (as identified by the exome analysis platform and manually confirmed by the variant interpretation team) to generate a GPT-4 query, asking which variant from the ML variant list causes the patient's phenotype based on the Human Phenotype Ontology terms originally used in the exome analysis.

Results: GPT suggested the true diagnosis in 30/43 (69.8%) of cases. Correct and incorrect diagnoses by GPT did not differ significantly in the presence of causative truncating/splice variants compared to missense/in-frame/synonymous variants ($p = 0.33$) or in the total number of gene publications in Pubmed ($p = 0.79$). However, correct diagnoses were associated with an earlier year of the first publication of gene association with human disease (median year: 2005 vs. 2015.5; $p = 0.0067$).

Conclusion: Our results support the use of GPT-4 as an added assistive tool in variant prioritization, yet its current limitations should be taken into consideration. Additional studies are needed to further characterize the advantages and limitations of GPT and other large language model-based tools in genomic medicine.

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P15.052.C Genomic approaches for solving rare and undiagnosed diseases through Rareboost project: results and insights

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Background: Rare diseases (RDs) impact millions worldwide, compounded by diagnostic uncertainties and limited treatment options. Many individuals endure years-long diagnostic odysseys, with some remaining undiagnosed. The RareBoost Project aims to bridge this gap by focusing on individuals with suspected but undiagnosed rare genetic diseases, leveraging translational research to enhance diagnostic accuracy and outcomes.

Material and Methods: The study cohort comprises 57 unresolved or undiagnosed cases selected from Rare and Undiagnosed Disease Advisory Board meetings, which bring together a multidisciplinary team of IBG researchers and clinicians from healthcare partners. Initial WES reanalyses on available data were performed on 39 of these cases, while WES, WGS, and/or RNA-Seq were conducted on 25 cases that remained without a definitive diagnosis.

Results: NGS analyses were conducted on 57 unresolved cases from 46 families enrolled in the RareBoost cohort. Genomic data reanalysis was performed for 39 patients, while WES (10/57), WGS (13/57), and RNA-Seq (3/57) were applied to the remaining undiagnosed cases to identify potential causative variants. Seven families (12%) achieved a definitive diagnosis based on pathogenic or likely pathogenic variants. Two cases involving synonymous variants were resolved through RT-PCR analysis and RNA-Seq analysis, respectively. Additionally, clinically relevant variants of uncertain significance (VUSs) were identified in 21 families (36.8%), with ongoing functional studies to assess their clinical relevance.

Conclusion: Our findings underscore the effectiveness of genomic approaches in diagnosing rare and undiagnosed cases, demonstrating their potential to enhance diagnostic accuracy and improve patient care.

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P15.053.D Identification of digenic interactions in autoinflammatory disorders by genetic analyses in large cohorts

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Background: The role of the digenic model in rare disorders has been relatively underexplored, mostly due to both the difficulties of statistically supporting these associations to disease and the limitations of generating experimental models. We have developed an approach based on the analysis of genomic data from hundreds of thousands of individuals available from large biomedical projects to detect or validate pathogenic digenic interactions.

Material and Methods: We analyzed genotyping data for 70 autoinflammatory disorder genes in UK Biobank for 467,000 European ancestry individuals based on PCA analyses. We compared the expected and observed frequencies of co-carriers for all possible pairs of variants from different genes.

Results: The analysis revealed candidate digenic interactions among AID genes, evidenced by deviations from neutrality of the genotypes distribution. One of the interactions, validated in an independent patient cohort, may provide insight into the incomplete penetrance of Familial Mediterranean Fever (FMF) where heterozygous individuals carrying pathogenic variants in the *MEFV* gene may or may not develop the disease. Specifically, we suggest that a genetic variant in a functionally related gene acts as a modifier, influencing disease expression by conditioning the action of the primary pathogenic variant in *MEFV*.

Conclusion: Our approach can identify digenic and modifier interactions in rare diseases. We have identified a candidate modifier gene that may explain incomplete penetrance in FMF.

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P15.054.A A combination of short-read and long-read sequencing elucidating the complex mechanism behind the gross genomic insertions in a variety of genetic disorders

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Background: Structural variants (SVs) are defined as genetic variants greater than 50 base pairs and include insertions, deletions, duplications, and rearrangements. SVs are a major source of genetic diversity associated with different diseases and phenotypes. The detection of gross insertions is challenging because the mutant allele exceeds standard NGS technologies read length.

Material and Methods: To identify the causative variants in rare disease patients, short-read sequencing methods (panel, exome, and genome sequencing) were applied in diagnostic setting. In cases where gross insertions were detected, confirmatory analysis comprising PCR and Sanger sequencing of the insertion/breakpoints was performed. Long-read sequencing (Nanopore) was applied in all confirmed cases to characterize the inserted sequence.

Results: Using short-read sequencing methods as first-tier, we identified gross insertions in 11 different genes (*SLC37A4, CLCN1, ATP7A, BCKDHB, ITGB3, NAGLU, LDLR, NF1, SLC26A3, PKLR* and *COL6A1*). The sequence of origin of the insertions was found to be: intron of another gene (1 gene), processed pseudogene (1 gene), mobile element (8 genes), and mitochondrial sequence (1 gene). The phenotype of the patients were matching to the genes associated disorders in which the insertions were found.

Conclusion: Our study underscores the utility of combined sequencing approaches in rare disease diagnostics. The results show that gross insertions can be detected in various genetic disorders and this mechanism is to be considered especially in previously unsolvable cases. Short-read sequencing plays a crucial role as first-tier in detecting these variants and long-read sequencing is a complementary method to characterize the mechanism of disease-causing insertions.

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P15.056.C Piloting BadgerSeq: A decentralized AI-assisted model for ultra-rapid long-read diagnostic genome sequencing

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Background/Objectives: Rare genetic disorders affect >20% of patients in level IV NICUs, where rapid genome sequencing is becoming the genetic test of choice. BadgerSeq is designed to improve availability, quality, and speed of sequencing, with a goal of 96 hours from clinical presentation to laboratory results.

Methods: BadgerSeq selects patients for sequencing prior to a genetics consult by evaluating all NICU patients daily using artificial intelligence (AI) software. FastHPOCR software text mines their EMR for HPO terms, which are input into the Mendelian Phenotype Search Engine, which ranks patients for sequencing. Infants whose scores exceed a threshold are offered rapid trio long-read Nanopore genome sequencing followed by AI-assisted tertiary variant analysis.

Results to Date: We are testing BadgerSeq's patient selection workflow on a retrospective cohort of 339 NICU patients, of whom 33% had genetic consults and 20% underwent rapid exome/genome testing. Initial results indicate that the workflow runs efficiently in a secure computing environment on a single virtual PC. We are piloting BadgerSeq's sequencing workflow using trios (proband + parents) from our Undiagnosed Disease Program. The first 10 cases averaged 46–48 hours from DNA to completion of initial tertiary analysis. The workflow correctly identified pathogenic SNVs and structural variants in the 3 positive controls and suggested candidate variants for 3 of 7 unknowns.

Conclusions: Our initial results demonstrate the feasibility of BadgerSeq's AI-assisted ultra-rapid sequencing workflow, a workflow that will offer hospitals a faster, more comprehensive long-read alternative to the current model of centralized high volume rapid short-read genome sequencing.

C.O.I. Disclosure:

M. Meyn: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Gene42. **J.M. Chen:** None. **D. Pavelec:** None. **B. Ross:** None. **J. Heilman:** None. **L.A. Frater-Rubsam:** None. **H.T. Nguyen:** None. **X. Shao:** None. **V. Horner:** None. **B.D. Webb:** None. **A.L. Hall:** None.

P15.057.D Accurate identification of pathogenic structural variants guided by multi-platform comparison

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Background: Structural variants (SVs) are a common cause of human diseases and contribute to inter-individual variability.

However, their detection remains challenging, especially when relying on short-read sequencing. To investigate and overcome these challenges, we combined an exhaustive selection of sequencing technologies in a cohort of nine individuals.

Material and Methods: We created a comprehensive SV detection benchmark to evaluate how each sequencing technology and SV caller perform across various settings as a guide for clinicians and researchers. Guided by our comparison, we designed a short-read-based machine learning model, dicast, to accurately identify true-positive (TP) SVs and demonstrate its applicability for clinical diagnostics.

Results: Our benchmark dataset includes 122,133 insertions, 104,099 deletions, 9,664 duplications, and 5,877 inversions. Dicast outperforms five leading short-read SV callers and consensus methods, identifying 79% more true-positive SVs than manta with comparable precision. It reduces false positives by 65% compared to the union of tested SV callers and increases true positives by 200% over consensus calling. We further demonstrate the clinical applicability of improved SV calling in three cohorts of rare disease patients with congenital limb malformations, neuromuscular disorders as well as atrial fibrillation.

Conclusion: While long-read-sequencing exhibits superior SV detection performance, short-read-sequencing remains more accessible, scalable, and less costly. We demonstrate that machine learning can push the boundaries of current short-read-based methods and dramatically reduces the need for time-consuming manual inspection of variants in the context of clinical diagnostics.

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P15.058.A Mapping global innovations in genomic newborn screening: A comparative review of technology and implementation

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Background: There are now over 30 research programs in genomic newborn screening (gNBS) worldwide, reflecting diverse strategies in approach, design, technology and implementation.

Project Name	Headquarters	Recruitment Goal	Status (Feb 2025)	Operational Model	Gene Content (Technology Backbone)	Sample	Genomic Test as first tier?
Anatolian Newborn Screening Program	Istanbul, Turkiye	10,000	Enrolling	Academic Multicenter	1393 (WGS)	Blood	Yes
Australian Targeted Genomic Newborn Screening Research Collaborative phase 2	Brisbane, Australia	60,000	Enrolling	Regional Public Health-Academic Multicenter	99 (Panel)	tNBS blood spot	Yes
BabyDetect	Liège, Belgium	40,000	Enrolling	Academic Single Center	405 (Panel)	Blood spot separate from tNBS	Yes
BabyScreen+	Melbourne, Australia	1000	Enrolling	Regional Public Health-Academic Multicenter	605 (WGS)	tNBS blood spot	Yes
BabySeq2	Boston, MA, USA	1000	Enrolling	Academic Multicenter	4299 (WGS)	Micropipette sample separate from tNBS	Yes
BeginNGS	San Diego, CA, USA	1,000,000	Enrolling	Academic Multicenter	>1500 (WGS)	Blood spot at same time, but separate from tNBS blood spot	Yes
Chen et al 2023	Shanghai, China	29,601	Post-enrollment	Academic Multicenter	142 (Panel)	tNBS blood spot	concurrent with tNBS
CRADLE	Bilthoven, Netherlands	under development	Pre-enrollment	National Public Health-Academic Multicenter	105 (Panel)	under development	Yes
EarlyCheck	Durham, NC, USA	10,000	Enrolling	State Public Health-Private-Academic Multicenter	188 (WGS)	tNBS blood spot	Yes

Project Name	Headquarters	Recruitment Goal	Status (Feb 2025)	Operational Model	Gene Content (Technology Backbone)	Sample	Genomic Test as first tier?
EpiGNs	Melbourne, Australia	100,000	Enrolling	Regional Public Health-Academic Multicenter	9 conditions (MS-QMA, methylation, WGS)	tNBS blood spot	No, low pass WGS as 3rd tier
EXAMEN	Moscow, Russia	7000	Post-enrollment	Academic Single Center	2350 (WES)	Umbilical cord blood sampling	Yes
FirstSteps	Athens, Greece	100,000	Enrolling	National Public Health-Private-Academic Multicenter	385 (WGS)	tNBS blood spot	Yes
Fecarotta et al 2025	Campagnia, Italy	108	Post-enrollment	Regional Public Health-Academic Single Center	105 (Panel)	tNBS blood spot	No, sequencing concurrent with confirmatory biochemical assay as 2nd tier
Generation Study	London, United Kingdom	100000	Enrolling	National Public Health Multicenter	463 (WGS)	Umbilical cord blood sampling	Yes
GenomaPuglia	Bari, Italy	3000	Enrolling	Regional Public Health-Academic Multicenter	407 (Panel)	tNBS blood spot	Yes
gnSTAR	Hangzhou, China	125,000	Post-enrollment	Academic Multicenter	154 (Panel)	tNBS blood spot	Yes
GUARDIAN Study	New York City, NY, USA	100,000	Enrolling	State Public Health-Academic Multicenter	450 (WGS)	tNBS blood spot	Yes
Lee et al 2019	Seoul, Korea	48	Post-enrollment	Academic Single Center	198 (Panel)	Blood spot separate from tNBS	Yes
NeoExome	Shanghai, China	3423	Post-enrollment	Academic Multicenter	601 (WES)	tNBS blood spot	Yes
NeoScreen	Queensland, Australia	under development	Pre-enrollment	Regional Public Health-Academic Single Center	under development	under development	Under development
NeoGen	Torino, Italy	4000	Post-enrollment	Academic Single Center	521 (WES)	tNBS blood spot	Yes
NeoSeq	Changzhou, China	196	Post-enrollment	Academic Single Center	136 (MTA-Seq)	tNBS blood spot	Yes
NESTS Phase 2	Beijing, China	11,484	Post-enrollment	Academic Single Center	465 (Panel)	Blood spot separate from tNBS	concurrent with tNBS
NewbornsInSA	Adelaide, Australia	1,000	Enrolling	Regional Public Health-Academic Multicenter	613 (WGS)	tNBS blood spot	concurrent with tNBS and Metabolomics
NEWBORN GEN SEQ TRAIL: NEWborn GENomic SEQuencing in	Sydney, Australia	1500	Enrolling	Regional Public Health-	605 (WGS)	Blood spot separate from tNBS	Yes

Project Name	Headquarters	Recruitment Goal	Status (Feb 2025)	Operational Model	Gene Content (Technology Backbone)	Sample	Genomic Test as first tier?
Screening: Therapy Ready And Information for Life Study				Academic Multicenter			
NEW LIVES: Genomic NEWborn screening programs - Legal Implications, Value, Ethics and Society	Heidelberg, Germany	under development	Pre-enrollment	National Public Health-Academic Multicenter	under development	under development	under development
PERIGENOMED Phase I	Dijon, France	2,500	Pre-enrollment	National Public Health-Private-Academic Multicenter	801 (WGS)	Blood spot at same time, but separate from tNBS blood spot	Yes
PREDiSPOSED Phase 1	Copenhagen, Denmark	2304	Pre-enrollment	Academic Multicenter	580 (Panel - Double Batch Sequencing Approach)	tNBS blood spot	Yes
Qataf Blooms	Doha, Qatar	4000	Pre-enrollment	Academic Single Center	114 (WGS)	Umbilical cord blood sampling	yes
Qingdao Newborn Genome Screening Program Phase 2	Qingdao, China	10,000	Post-enrollment	Academic Multicenter	234 (WGS)	Blood spot separate from tNBS	Yes
ScreenPlus	Bronx, NY, USA	100,000	Enrolling	State Public Health-Academic Multicenter	14 (Panel)	tNBS blood spot	No, sequencing of relevant gene only as 3rd tier
Screen4Care	Ferrara, Italy	25,000	Enrolling	National Public Health-Private-Academic Multicenter	245 (Panel)	dried blood spot (separate from tNBS)	Yes
Wang et al 2023	Nanjing, China	10,334	Post-enrollment	Academic Single Center	164 (Panel)	Blood spot at same time, but separate from tNBS blood spot	concurrent with tNBS

We highlight key regional initiatives and methodological differences.

Material and Methods: We compare and contrast the different approaches undertaken in implementing gNBS, including operational models, gene content, technology backbone and methodologies for consent, returning results and long-term follow up. Data have been compiled from personal communications, International Consortium on Newborn Sequencing conference proceedings and published literature.

Results: For the 33 programs with information available, genes assessed vary from 14 to 4299 (median 405; mean 592) on varied technology backbones [WGS = 13, targeted NGS panel = 13, WES = 3, other = 2, under development = 2], with majority using genomic testing (28) as a first-tier test. Some programs are focused on developing companion digital tools for education and consent (7), and studying metabolomics (1) and epigenomics (1). Operational models include Academic (16), Public Health (1), Public Health-Academic (12) and Public Health-Private-Academic (4).

Conclusion: The global landscape of gNBS is marked by a rich diversity of scientific approaches, technologies, operational models and implementation strategies. Each region's approach reflects its unique healthcare priorities, resource availability, and ethical considerations, clearly indicating that one size does not fit all. The current scarcity of gNBS outcome data, essential for defining best practices, also explains the diverse approaches. Future research and policy development should prioritize the creation of flexible, sustainable models that allow for localized modifications and drive equitable health outcomes.

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P15.059.B Deciphering the expression signature in Kleefstra Syndrome by RNAseq

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Baldo[3], Alessia Arado[3], Michela Mura[4], Camilla Meossi[5], Giulia B. Marchetti[4], Donatella Milani[4], Valentina Massa[2,6], Cristina Gervasini[2], Maria Iascone[1]

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Background: Haploinsufficiency of *EHMT1* is the leading cause of Kleefstra syndrome (KLEFS, OMIM #610253), a rare condition characterized by severe neurodevelopmental disorder and multisystemic involvement. Large or intragenic deletions, splicing, and truncating variants are the most common causes of KLEFS, but pathogenic missense variants are also reported and tentatively associated with a milder phenotype. We aim to characterize the expression pattern of *EHMT1* and related pathways in patients with KLEFS to deepen our understanding of the pathogenetic mechanism and attempt a genotype-phenotype correlation.

Material and Methods: Patients were enrolled in the setting of a multicenter study. Genetic data and blood samples were collected. Lymphoblastoid cell lines (LCLs) were obtained from blood cells of patients with different pathogenetic variants and used to perform RNAseq. We obtained and analyzed 18 KLEFS LCLs and compared them to 10 healthy controls. Relative *EHMT1* expression was calculated by comparing the normalized TPM (transcripts per million) value of *EHMT1* and housekeeping genes. Edger and DESeq2 assessed differential expression patterns, and enrichment analysis was performed using the ORA (over-representation analysis) method.

Results: The expression levels of *EHMT1* are significantly reduced in KLEFS LCLs, with slight differences among different types of mutations. We identified three impaired pathways involved in neuronal homeostasis and extracellular matrix interaction.

Conclusion: Our preliminary results catch a glimpse on the biological effects of different *EHMT1* defects, possibly unravelling new genotype-phenotype correlations and identifying novel candidate targets for future therapies.

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P15.060.C Development of iPSC-derived vascular smooth muscle cell-model for Loeys-Dietz syndrome type V aortic phenotype

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Background: Thoracic aortic aneurysm and dissection (TAAD) is a hallmark of Loeys-Dietz syndrome (LDS) and a prominent cause of sudden death. Unfortunately, the pathophysiology of TAAD remains largely elusive which complicates therapy development. A human in vitro model that closely recapitulates the disease phenotype is required to gain better insight into the pathomechanisms underlying TAAD as well as to expedite the development of new treatments.

Material and Methods: In this preliminary study, induced pluripotent stem cell (iPSC)-derived neural crest-vascular smooth muscle cell (NC-VSMC) lines from two TAAD-presenting LDS type V (LDS-V) patients carrying a likely pathogenic p.(Asp263His) variant in TGFB3 and a healthy control were generated and characterized at transcriptional and translational levels.

Results: Consistent with previous biochemical analysis of LDS-V aortic tissue, we observed a trend towards reduction in contractile marker expression along with reduced contractility and calcium flux upon stimulation with the thromboxane A2 receptor agonist U46619 in patient versus control NC-VSMCs. Additionally, we observed upregulation of the transforming growth factor beta (TGFβ) pathway, a well-known contributor to syndromic TAA. Interestingly, the cellular phenotype differed between the NC-VSMCs of the two patients, with one patient exhibiting a more deteriorated cellular phenotype, aligning with his more severe TAAD phenotype.

Conclusion: For the first time we describe iPSC-VSMC modelling of LDS-V. The obtained data suggest that iPSC-derived NC-VSMCs from LDS-V patients recapitulate at least part of the disease phenotype. Upon consolidation of our findings in additional NC-VSMC clones, we deliver the TAAD community a novel tool for preclinical LDS-V research.

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P15.061.D The homozygous *FICD* p.(Arg371Ser) variant causes a syndrome of neonatal diabetes, cataracts and developmental delay through a variant-specific mechanism

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Background: Variant-specific disease mechanisms, where different variant(s) in the same gene cause different disorders, are increasingly being identified in human monogenic disease. We recently identified a homozygous *FICD* variant, p.(Arg371Ser), in 5 individuals with neonatal diabetes (NDM), cataracts and developmental delay (DD). We hypothesised that this phenotype was variant-specific as a different recessive *FICD* variant, p.(Arg374His), causes spastic paraplegia-92. The clinical features of spastic paraplegia-92 did not overlap with those in our 5 cases. Biochemical studies predicted both variants to similarly affect *FICD*'s function. It is therefore unclear how these variants cause distinct phenotypes. Here, we further explore whether the *FICD* p.(Arg371Ser) variant causes NDM in a variant-specific manner.

Material and Methods: We screened for rare (GnomAD MAF <0.01%) biallelic *FICD* variants in 384 probands with diabetes diagnosed <3years of unknown genetic cause using genome sequencing.

Results: We identified four additional individuals homozygous for the *FICD* p.(Arg371Ser) variant, taking the total to 9. 8/9 (including 5/5 original cases) had the same nucleotide change (NM_007076.2:c.1113G>C). Importantly, the ninth case harboured a different nucleotide change (NM_007076.2:c.1113G>T). Clinically, age at diabetes onset ranged between 12 weeks and 2 years (median = 29.5 weeks). Early-onset cataracts (4/9) and DD (5/9)

were common features. No spastic paraplegia-92 features were reported.

Conclusion: Identification of the *FICD* p.(Arg371Ser) variant in nine individuals, including one with a different nucleotide change, strongly supports that this variant causes NDM, cataracts and DD via a variant-specific mechanism. Further studies are needed to understand the mechanisms underlying the specific phenotypes caused by *FICD* variants.

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P15.062.A CLINICAL, DEMOGRAPHIC AND GENOTYPE SPECIFIC ASSOCIATIONS IN HERITABLE TP53-RELATED CANCERS

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Background: Heritable *TP53*-related cancer (h*TP53*rc) syndrome is an autosomal dominant disorder caused by germline *TP53* variants, leading to the early onset of multiple cancers. Essential genotype-phenotype data is missing to improve h*TP53*rc management guidelines. This study aims to provide a comprehensive genotype-phenotype analysis to deliver clinical-, demographic- and genotype-specific associations.

Material and Methods: We curated the germline dataset from International Agency for Research on Cancer TP53 database (R20). For genotype-phenotype analysis, we: (1) considered families with at least one confirmed germline carrier; (2) standardized phenotypes; (3) classified variants regarding pathogenicity (ACMG) and predicted functional impact (dominant-negative effect [DNE] and loss-of-function [LOF]). Statistical analysis were performed with RStudio.

Results: The curated dataset comprised 2.733 phenotypes from 2.071 individuals (62.7% females), from which 34.3% developed the first cancer between 0–17 years, while 14.5% were diagnosed after 50. Phenotypes were grouped into 24 cancer-types associated with 202 pathogenic/likely-pathogenic variants (55.4% DNE_LOF, 13.7% notDNE_LOF, and 30.9% unkownDNE_-LOF). Three major female cancer-susceptibility profiles were identified: **(1)** variants in codon-133 were associated with breast carcinomas between 18–49 years ($OR = 3.8$, $p = 0.010$); **(2)** c.824G>A variant was associated with soft-tissues sarcoma ($OR = 6.5$, $p < 0.001$); **(3)** c.1010G>A variant was associated with adrenal gland carcinomas, between 0–17 years ($OR = 23.4$, $p < 0.001$). Additionally, patients carrying DNE_LOF variants with a first diagnosis between 0–17 years were 2.3-fold more likely to develop multiple malignancies than notDNE_LOF ($p = 0.007$). Lastly, variants c.659A>G and c.844C>T were significantly associated with lower age-of-diagnosis.

Conclusion: This genotype-phenotype analysis provides the rationale and context to prioritize intensive surveillance and/or prophylaxis across hTP53rc patients.

C.O.I. Disclosure:

A. Dias: None. **R. Matos:** None. **R. Quental:** None. **L. F. Maia:** None. **C. Oliveira:** None.

P15.063.B A prediction of the economic impact of whole-genome sequencing on the diagnostic pathway of paediatric rare genetic diseases: a multi-centre analysis

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Background: Diagnosing rare genetic diseases in children involves prolonged, costly pathways with low diagnostic yield (DY). This analysis evaluates comprehensive genomic testing versus traditional diagnostic approaches in paediatric rare diseases.

Material and Methods: Data were collected in 2023–24 at two Italian and one Spanish centres through semi-structured interviews mapping and costing two patient settings: hypotonic inpatients in Intensive Care Units (ICUs) receiving genetic testing, outpatients with suspected neurodevelopmental disorders facing prolonged diagnostics. Incremental costs for diagnosed patient (IC: cost to reach a diagnosis divided by DY of technique used) were compared across past (without clinical exome sequencing [CES], Whole-Exome Sequencing [WES] or WGS), current (CES, WES, WES+Chromosomal Micro Array), and future (WGS) scenarios.

Results: Across centres, ICU costs (limited to pre-diagnosis ICU days) constituted up to 99% of pre-diagnosis inpatients expenses in all scenarios, while genetic testing was a minor component. As diagnostic techniques advanced, DY increased (e.g., past-15%, current-50% predicted-65%) while pre-diagnosis days in ICU and relative costs-to-diagnosis decreased (e.g., from 180 to 20 and 12 days, from € 297k to € 32.2k and €19.8k). For outpatients, DY similarly rose (e.g., from 19% to predicted 60%) with expected moderate cost increases in future. Overall, IC is expected to decrease as techniques evolve (e.g., inpatients: €2,007k to €72.4k and €38.4k; outpatients: €27.6–19.9k to €9.5k and €8.5k).

Conclusion: CES and WES are routine in all centres, with WGS anticipated to follow. WGS is predicted to improve DY, reduce IC, shorten diagnostic time, improving care for children with rare genetic diseases.

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P15.064.C Phenotypic and genotypic comparison between severe forms and microform of holoprosencephaly in a cohort of 173 individuals with pathogenic variants in *SHH* gene

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Background: Holoprosencephaly (HPE) is a congenital condition caused by incomplete cleavage of the cerebral hemispheres during embryonic development, resulting in a range of cerebral and craniofacial abnormalities. This study compares the differences between severe (alobar, semilobar, lobar) forms and microforms of HPE in individuals with pathogenic variants in the most frequently gene implicated in HPE, *SHH*.

Material and Methods: The data of 173 individuals (77 probands, 96 relatives) were collected from HPE database which lists patients suffering from HPE in Europe and for which advice has been requested in Rennes University Hospital, France.

Results: 47.4% of individuals exhibited microforms of HPE, while 18.5% had alobar forms, 9.2% semilobar forms, 5.2% lobar forms, and 9.8% were asymptomatic (relatives). The analysis showed that severe forms of HPE were significantly associated with higher rates of brain, adrenal and craniofacial anomalies compared to microforms ($p < 0.05$). In contrast, dental malformations and nasal cavity abnormalities were more frequent in microforms ($p < 0.01$). Importantly, in a specific region of the *SHH* gene (amino acids 275–330), we didn't found any pathogenic variants, although benign variants have been described, which suggests that variant in this area may not be pathogenic.

Conclusion: These findings emphasize the distinctive clinical features of microforms compared with severe forms and broaden the spectrum of malformations associated with HPE related to *SHH* (adrenal malformations, over-representation of dental and nasal cavity abnormalities in microforms). The study highlights the complexities of genetic counseling in HPE: intra- and inter-familial variability, intellectual disability even in microforms (38%), and oligogenism.

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P15.065.D Telomere biology disorders- clinical service, dilemmas and challenges

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Background: Germline variants affecting telomere maintenance cause Telomere Biology Disorders (TBDs). Adult-onset TBDs include pulmonary fibrosis, bone marrow and liver diseases. Lack of clinical guidelines may delay diagnosis and tailored management in late-onset TBDs. We describe our experience in a novel TBD clinic.

Material and Methods: Cohort analysis of patients referred to the TBD clinic at the Genetics Institute at the Tel-Aviv Medical Center from February to December 2024. Indications for referral, phenotypes and diagnostic tests, including telomere length and sequencing results, are reviewed.

Results: Twenty-four probands were referred to our clinic. Average age at first visit was 56 years (range 3–81 years), 17/24 (70.8%) were familial and 7/24 (29.2%) were simplex cases. Ethnic origin was Jewish in 22/24 (91.7%). Two probands were from consanguineous non-Jewish families. Phenotypes included pulmonary fibrosis in 20/24, (83.3%), myelodysplasia in 2/24 (8.3%), liver fibrosis in one (4.2%), and multisystem disease in one (4.2%). Compared to controls, 20/21 (95%) showed short telomeres, 4 of whom measured in the extremely short range. Variants in TERC, TERT and MDM4 confirmed TBD in 5/7 (71.4%), who performed genetic testing. Diagnostic challenges and management dilemmas included negative next-generation-sequencing in familial pulmonary fibrosis, variant interpretation, selection of bone marrow donors in a familial TERT-related TBD, and follow-up of first-degree relatives in TERC-related TBD.

Conclusion: TBDs are complex disorders. A collaborative multidisciplinary approach is vital in management of patients. The advent of available precision medicine mandates their early diagnosis. Further studies will help to establish clinical guidelines and healthcare public funding.

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P15.066.A Concordant epigenetic and discordant clinical iPPSD3 manifestations in two monozygotic adolescent twins

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Background: iPPSD3, formerly pseudohypoparathyroidism 1B, is characterized by PTH resistance, hypocalcemia, hyperphosphatemia and, sometimes, TSH resistance. It is caused by epigenetic variants occurring in one or more *GNAS*-DMRs, with consistent hypomethylation at *GNAS A/B*:TSS-DMR. We describe monozygotic 14-year-old twins, one with clinical features suggestive of iPPSD3 and the other with unaltered phosphocalcic metabolism.

Material and Methods: Analysis of the *GNAS* locus by MS-MLPA (ME031 kit; lot: C1-0523) was performed on saliva and blood samples from both twins. Uniparental disomy of chromosome 20q was assessed through SNParray, a custom MS-MLPA (targeting *GNAS, L3MBTL1, NNAT* and *MCTS2P* DMRs) and microsatellite typing. Finally, genome-wide by long-read sequencing was performed on blood and saliva samples.

Results: MS-MLPA revealed similar partial methylation disturbance for all *GNAS*-DMRs in both twins´ blood samples, whereas the healthy sister´s saliva suggested fewer epigenetic alterations. The long-read sequencing study, which does not modify or digest the DNA, and performs control-independent analysis, revealed the same methylation pattern in both blood samples, but different at maternal alleles in saliva (Table).

Methylation percentages detected by long-read sequencing									
		GNAS-NESP:TSS-DMR		*GNAS-AS*:TSS-DMR		*GNAS-XL*:TSS-DMR		*GNAS A/B*:TSS-DMR	
		Blood	Saliva	Blood	Saliva	Blood	Saliva	Blood	Saliva
Control	Paternal allele (%)	100	100	0	0	0	0	0	0
	Maternal allele (%)	0	0	100	100	100	100	100	100
Affected twin	Paternal allele (%)	100	100	0	0	0	0	0	0
	Maternal allele (%)	40	65	40–50	0–25	50	25	50	25
Healthy twin	Paternal allele (%)	100	100	0	0	0	0	0	0
	Maternal allele (%)	25	50	25–50	75	50	75	50	50

Conclusion: Given the clinical discordance and the epigenetic findings, we postulate that the epigenetic alteration occurred postzygotically, but before zygote cleavage. Probably, the altered cells in each of the sisters migrated to different embryonic cell lines, involving those related with phosphocalcic metabolism in the affected twin but not in her sister.

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P15.067.B Application of polygenic risk scores for Latvian hypercholesterolemia patient differentiation

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Background: Polygenic hypercholesterolemia (PH) comprises about 30% of patients with a clinical diagnosis of familial hypercholesterolemia (FH). In our recent study, we applied the published LDL-C PRS (PGS000115) to explore the genetic background of Latvian patients with clinical FH diagnosis. The chosen score had overall good discriminatory ability. Nevertheless, despite the availability of pre-existing PRS models for the LDL-C trait, selecting the most fitting score for a specific population, even with European origin, remains a challenge.

Material and Methods: The DNA from 329 adults with clinical FH diagnosis and 515 controls from the Latvian reference cohort was sequenced using the MGI DNBSEQ-T10×4RS platform. The PRS was calculated using 118 publicly available LDL-C PRS models from the PGS Catalog.

Results: Ninety-seven PRS met the minimum match rate threshold of 0.75. The PGS004793 model reached the highest area under the curve (AUC = 0.82) among models developed in European ancestry cohorts and the third highest overall. There was a positive correlation between the number of SNPs included in a model and AUC ($p = 0.006$). The PGS000115 and PGS004793 differentiated FH and non-FH groups, but PGS004793 showed stronger discriminatory ability and less variability in PRS distributions. The proportion of FH patients decreased with higher PRS percentiles, with PGS004793 displaying more pronounced differences between LDL-C PRS quartiles.

Conclusion: The number of SNPs positively correlated with PRS model performance. While both models performed well, PGS004793 explained 40.08% of PH inheritance in non-FH cases, compared to19.84% by PGS000115, making it more effective model.

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P15.068.C miRNA expression profile in symptomatic and presymptomatic genetic FTD

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Background: Frontotemporal dementia (FTD) is a neurodegenerative disease characterized by significant clinical and genetic heterogeneity, with approximately 40% of cases linked to hereditary genetic mutations, including MAPT, GRN, and C9ORF72. Recently, microRNAs (miRNAs) have emerged as key regulators of cellular processes related to neurodegeneration and as potential biomarkers for FTD. However, their relevance in presymptomatic stages remains poorly understood.

Material and Methods: We conducted a miRNA expression analysis using TaqMan OpenArray® panels on blood samples collected from 171 individuals, including symptomatic mutation carriers (SMC), presymptomatic carriers (PMC) and healthy non carriers (NC). Dysregulated miRNAs were validated and further investigated.

Results: In C9ORF72, miR-20b-5p and miR-223-5p were significantly upregulated in SMC (fold regulation over NC: 2.418 $p = 0.0336$ and 7.829 $p < 0.0264$ respectively), and PMC (5.518, $p < 0.0001$ and 3.941, $p < 0.0001$ respectively). In GRN mutation carriers, miR-28-3p was altered in both SMC and PMC (fold regulation over NC: 1.484 $p < 0.050$ and 3.287, $p < 0.050$). In MAPT mutation carriers, miR-28-5p, miR-192-3p, miR-25-3p, and miR-532-3p were altered only in SMC (fold regulation over NC: 1.496 $p < 0.050$, 1.911 $p = 0.006$, 1.468 $p < 0.05$ and 0.728 $p < 0.05$). Bioinformatic analysis revealed enrichment of pathways related to neurodegeneration and synapse impairment.

Conclusion: These results suggest that miRNA expression levels are de-regulated in mutated SMC, and also in *C9ORF72* and *GRN* PMC. Notably, miR-20b-5p, miR-223-5p, and miR-28-3p were increased in preclinical stages of the disease, supporting their role as early biomarkers for *C9ORF72*-FTD and *GRN*-FTD. Conversely, alterations in *MAPT* carriers appeared only in symptomatic stages, suggesting a different involvement in disease progression.

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P15.071.B Proteomics analysis in a diverse cohort of 50 unsolved cases with suspicion of a rare genetic disorder

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Background: Rare diseases, approximately 80% of which are genetic, pose a significant burden on the healthcare system. Despite advancements in diagnostic methods, particularly next-generation sequencing, many cases remain unsolved.We aimed to use a multi-omics approach, mainly transcriptomics and proteomics, to investigate unsolved cases with a suspicion of a genetic disorder.

Material and Methods: Our cohort consisted of 50 unsolved cases with heterogeneous phenotypes, including neurodevelopmental disorders with variable severity (31/50, 62%), epilepsy (7/50, 14%), suspicion of mitochondrial disorder (7/50, 14%), and other clinical presentations. The median age of individuals was 8 years, ranging from 1 to 63 years. The male-to-female ratio was roughly equal, with 28 (56%) male participants. All patients had undergone exome sequencing (mostly trios 44/50, 88%) but remained unsolved. For the proteomics, we used cultured skin fibroblasts, and the results were bioinformatically analyzed using the PROTRIDER pipeline. We combined different multi-omics results manually.

Results: Overall, the PROTRIDER pipeline identified 442 proteins with aberrant expression (adjusted p-value < 0.1), of which 256 (57.9%) showed reduced expression (fold-change <1). Among the proteins with significantly low expression, three cases were particularly relevant to the phenotype, but no corresponding DNA variants were identified.

Conclusion: Despite employing a multi-omics approach, no definitive disease-causing variants were identified in our unsolved patients' cohort after proteomics analysis. We believe proteomics is more effective in cases with well-defined and specific phenotypes. The diversity and ambiguity of our cohort may have limited the approach's effectiveness.

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P15.072.C Uniparental isodisomy as a mechanism of inheritance of autosomal recessive diseases

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Background: Uniparental disomy (UPD) is a rare pathology in which diploid offspring carry a chromosome pair from one parent. We report cases of uniparental disomy leading to diseases with a traditionally autosomal recessive type of inheritance.

Material and Methods: Sequencing of targeted panels "Ophthalmo", "Limb-Girdle Muscular Dystrophies", "Clinex", including genes responsible for various hereditary pathologies. Validation of variants was performed by Sanger sequencing of the *RPE65, SGCD, PREPL* genes. Analysis of the copy number of the corresponding genes using MRC-Holland kits. Relationship was confirmed by analyzing microsatellite markers on 12 different chromosomes. Microsatellite marker analysis of the studied gene region on chromosomes 1, 2 and 5.

Results: In four patients with referral diagnoses of LCA (2 probands), limb-girdle muscular dystrophy and congenital myasthenia, pathogenic variants were identified in the *RPE65, SGCD* and *PREPL* genes, respectively, in a homo-/hemizygous state. When determining zygosity, it was found that one of the patient's parents did not have a pathogenic variant in the studied gene. Two copies of the corresponding genes were detected in the probands and their parents. Relationship was confirmed in all families. To prove the hypothesis of UPD, an analysis was performed using polymorphic markers from the studied gene region. It was found that each of the probands lacked alleles of one of the parents, which indicates UPD for the chromosomal region containing the studied genes.

Conclusion: The established mechanism of inheritance is an important step towards accurate medical genetic counseling.

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P15.073.D Individualized genetic testing of patients with renal and cardiac diseases at the University of Debrecen

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Background: Inherited renal and cardiac diseases are clinically and genetically heterogeneous. Next-generation sequencing (NGS) studies have previously described several mutated genes in the background of these diseases. Therefore, two targeted NGS gene panels have been established in our department to identify mutations in the affected patients.

Patients and Methods: We have tested patients with suspected genetic kidney (n = 33) and heart (n = 49) diseases. The nephrology (n = 33) and cardiology (n = 43) genes were sequenced using NGS method. All small-scale mutations were confirmed by Sanger sequencing. Copy number variations (CNVs) were confirmed using multiplex ligation-dependent probe amplification (MLPA).

Results: NGS gene panel analysis showed 39% positivity rate (n = 13) in case of patients with renal disorders and 18% diagnostic efficiency (n = 9) in patients with cardiac diseases, which correlates with the international data. We found genetic alterations most frequently in the *PKHD1* (n = 5) and *COL4A3* (n = 4) genes using Nephrology NGS gene panel. Four patients had mutation in other genes. The most common mutation type was missense (n = 9), followed by three cases of CNVs and nonsense mutations (n = 2). The most prevalently mutated gene was *TTN* (n = 2) in the patients with hereditary cardiac disease. In addition, causative mutations were detected in the following genes: *ACTC1*, *DSG2*, *FLNC*, *KCNQ1*, *MYBPC3*, *PLN* and *TNNI3*. In these, we identified mainly missense (n = 3), nonsense (n = 2),

frameshift (n = 2), splicing (n = 1) and synonymous (n = 1) mutations.

Conclusion: The early indication of genetic testing in patients with unusual or progressive renal and cardiac disease can improve the diagnostic efficiency, and may influence the therapeutic decision making.

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P15.074.A The potential utility of WES in newborn screening workflow

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Background: Newborn Screening(NBS) is a major Italian public health program to screen all newborns to prevent the long-term consequences of inborn errors of metabolism (IEMs). Immunochemistry and tandem-mass spectrometry are currently the gold-standard methods for NBS, but false-positive cases are still common (0.3%/year). We evaluated the feasibility of including next-generation sequencing(NGS) in NBS workflow to reduce the number of false positives.

Material and Methods: We perfomed Whole Exome Sequencing(WES) on genomic DNA extracted from dried blood spots(DBS) of 39 newborns positive to biochemical first-tier-test of NBS and referred to clinical evaluation. Libraries were prepared with "Illumina DNA Prep with Exome 2.5 Enrichment" and sequenced on NextSeq2000 (Illumina). Analysis was performed first using a virtual panel of 125 genes related to IEMs. Whole exome was analyzed in negatives or heterozygous cases. Variants were classified according to ACMG criteria.

Results:20/39(51.3%) cases had no variants in genes implicated in metabolic diseases; 13/39(33.3%) were heterozygous and 3/39(7.7%) compound heterozygous,3/39(7.7%) were homozygous for variants in ACADVL, ACADS and GALE genes. Variants in ACADVL and ACADS are pathogenic, while GALE variant is likely benign, more common among African-American populations and associated with a mild-form of epimerase deficiency galactosemia.

Conclusion: These results show that WES introduction in NBS workflow reduce the number of false positives and then patient's number monitored by clinicians. Only 2/39 cases, with this approach, would have been referred to clinical centers, while heterozygous could have followed another clinical pathway, without the need for immediate intervention, reducing costs and parental anxiety and allowing more efficient care management.

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P15.075.B Expanding the diversity of genetic mechanisms in rare diseases through Long-Read sequencing

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Background: Next-generation sequencing methods have revolutionized genetic diagnosis, although they present certain limitations due to short reads. We explored the strengths of long-read sequencing with nanopore in rare diseases.

Material and Methods: Long-range (LR) PCRs were barcoded and sequenced in MinION. Whole genome or targeted sequencing of unmanipulated DNA was done in MinION or PromethION. Sniffles, Clair3 and modkit, were used to identify structural variants (SVs), SNVs or epigenetic changes.

Results: 1) Identification of founder effect. LR-PCRs and sequencing of *SERPINC1* in 14 patients with antithrombin deficiency revealed a common intragenic haplotype with 13 SNVs linked to the p.Thr147Ala variant. 2) Characterization of SVs. Nanopore sequencing fully characterized 12 SVs affecting *SERPINC1* or *F11* in patients with antithrombin or FXI deficiency, regardless their size (198 bp-5 Mb), or type (deletion, duplication, inversion, insertion). It defined the length and the breakpoints, which involved repetitive elements, and resolved complex SVs, which were incorrectly classified by other methods. 3) Identification of the molecular basis. Inversions, complex rearrangements or retrotransposon insertions were identified in candidate genes of 12 patients lacking a molecular diagnosis for 7 different rare diseases. 4) Identification of new mechanisms. Expansion of a ***CALR*-mutated** myeloid clone with epigenetic silencing of the wild-type allele in a woman heterozygous for the *PGK1*^{Ser320Asn} variant explained her anemia.

Conclusion: Nanopore sequencing opens new perspectives in the genetic diagnosis of rare diseases, enabling: 1) rapid and cost-effective haplotype resolution; 2) identification and characterization of SVs; and 3) detection of new pathogenic mechanisms (retrotransposon insertions, inversions, and epigenetic modifications).

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P15.077.D Real-world genetic prevalence estimates of Factor V deficiency using large-scale genomic data

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Background: Factor V (FV) deficiency is a rare autosomal recessive bleeding disorder characterized by reduced levels and/or activity of FV coagulation factor in blood. Its estimated prevalence is 1:10^6. Currently, >220 causative mutations have been identified throughout the *F5* gene.

Material and Methods: We explored exome/genome data from >800,000 subjects in the genome Aggregation Database (gnomAD v.4.1). Worldwide and ethnic-specific prevalences were calculated with the Genetic Prevalence Estimator (GeniE, https://genie.broadinstitute.org/).

Results: A total of 6,228 *F5* variants were identified in gnomAD; of these, the number labelled as disease-causing depended greatly on the specific criteria used for selection. Three datasets were generated: a "GeniE recommended/default" dataset, consisting of 377 variants, and two custom datasets, in which pathogenic missense variants were defined either on REVEL >0.773 or AlphaMissense score. These included 656 and 742 variants, respectively. The GeniE default dataset gave a worldwide prevalence estimate similar to what expected (1:10^6), whereas the custom datasets both gave higher prevalence (5:10^6 and 5.8:10^6). Interestingly, by using as benchmark 226 known FV deficiency-causing variants annotated in the Human Gene Mutation Database (HGMD), we found that the custom datasets both had a much similar distribution to HGMD set in terms of representation of different variant types compared to the default GeniE dataset ($p = 0$), which is inflated with loss-of-function variants.

Conclusion: We provide a critical assessment of methods to select potentially pathogenic variants from population data, which can be adapted to other rare diseases. Our results suggest that FV deficiency prevalence might be higher than previously thought.

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P15.078.A Bridging gaps in diagnosis: the high impact of gene-disease relationship evaluation

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Background: One of the primary challenges faced by diagnostic laboratories is evaluating the clinical significance of genetic variants. A crucial part of this process involves establishing gene-disease relationships (GDR). In this study, we summarize our experiences over the past two years using the ClinGen framework in the daily operations of a diagnostic laboratory.

Material and Methods: The ClinGen Clinical Validity Framework was used for evaluating GDR. We systematically assessed genes without OMIM entries or those with provisional entries. Gene selection was based on specific variants identified during routine diagnostic evaluations of patients' genomic data. The internal CENTOGENE biodatabank, containing exome/genome data from previously tested individuals, alongside data from literature and public databases, was utilized for gene classification. The framework allows for classification into definitive, strong, moderate, limited, or no known disease relationships.

Results: Over 700 genes were assessed, resulting in a strong/definitive level of evidence for 22% of genes, moderate for 25%, limited for 44%, and no known disease relationship or disputed for 9% of the genes. A total of 104 genes (14%) without OMIM entries achieved moderate or strong GDR.

Conclusion: Our findings highlight the importance of thoroughly assessing gene clinical validity data and utilizing genetic data repositories. The implementation of the ClinGen standardized scoring system for routine gene-disease association assessments is straightforward to apply and highly relevant in a clinical diagnostic context, providing benefits for patients, families, and healthcare providers.

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P15.079.B Diagnostic yield of whole exome sequencing versus whole genome sequencing in pediatric patients with rare phenotypes: a systematic review and meta-analysis

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Background: Rare diseases affect 6% of Western societies and are a leading cause of pediatric mortality. The popularization of Next Generation Sequencing technologies, specially whole exome sequencing (WES), revolutionized the diagnosis of children with rare disease. Still, most patients face extensive diagnostic odysseys and remain undiagnosed. Recently, whole genome sequencing (WGS) emerged in hope that its broader coverage could improve diagnostic yield compared to WES. This study aims to systematically review and meta-analyze the diagnostic power of WES versus WGS in pediatric populations with rare diseases.

Methods: A systematic review of PubMed, Cochrane, and Embase databases was performed on December 11, 2024, for non-randomized studies comparing WGS diagnostic yield with WES or WES reanalysis in pediatric populations with rare disease. Statistical analyses were performed in R software version 4.3.2. and the study was registered on PROSPERO (CRD42024619640).

Results: In a cohort of 1,767 patients from 12 studies, WGS-specific diagnostic yield was 8.2% (95% CI: 5.1%–13.0%; $p < 0.0001$). Subgroup analysis revealed that WES reanalysis and WGS provided statistically similar diagnostic yields in patients with prior negative WES due to overlapping confidence intervals. The diagnostic rate of WES reanalysis was 14.3% (9.1%–21.7%; $p < 0.0001$), while WGS was 7.8% (5.3%–11.3%; $p < 0.0239$).

Conclusion: This meta-analysis showed that WGS could establish molecular diagnoses in 8.2% more patients after negative WES. However, similar diagnostic yields from WES

reanalysis and WGS emphasize the importance of periodic reanalysis and variant reinterpretation in diagnostic workflows.

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P15.080.C Unraveling the Genetic Landscape of Progressive Familial Intrahepatic Cholestasis: Novel Variants and Functional Insights

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Background: Progressive Familial Intrahepatic Cholestasis (PFIC) encompasses a heterogeneous group of rare, inherited liver disorders characterized by impaired bile flow due to genetic defects affecting hepatocellular transporters, bile canaliculi structure, or cholangiocyte function. Despite the identification of 13 PFIC types (ATP8B1, *ABCB11*, and *ABCB4*, while more recently discovered causative genes include *TJP2*, *NR1H4/FXR*, *SLC51A*, *USP53*, *KIF12*, *ZFYVE19*, *MYO5B*, *SEMA7A*, *VPS33B* and *PSKH1*), primarily linked to mutations in genes regulating bile acid homeostasis, approximately 30% of cholestatic cases remain genetically undiagnosed, highlighting the need for advanced investigative approaches.

Material and Methods: We analyzed 40 pediatric patients (average age of onset 0.8 years) with intrahepatic cholestasis through whole exome sequencing (WES). Clinical manifestations included hepatomegaly, jaundice, pruritus, and various extrahepatic signs. We performed comprehensive genetic analysis through trio-based WES, extending analysis beyond established PFIC genes.

Results: Beyond identifying variants in known PFIC genes (*ATP8B1*, *ABCB4*, *MYO5B*), we discovered novel variants in previously unreported genes, including *PLEC* (affecting BSEP targeting), *NEK8* (associated with bile duct paucity). Functional studies validated the pathogenicity of identified variants and their impact on bile acid transport pathways.

Conclusion: This multi-faceted approach will advance our understanding of cholestatic disorders by combining genetic discovery with functional validation, potentially leading to improved diagnostic strategies and personalized therapeutic approaches.

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P15.081.D Whole Genome and RNA sequencing, a double approach to complex clinical cases: the Shwachman-Diamond example

Lucrezia Goisis[1], *Camilla Lucca*[1], *Lidia Pezzani*[2], *Laura Pezzoli*[1], *Daniela Marchetti*[1], *Matteo Bellini*[1], *Francesca Cappuccini*[1], *Chiara Mongodi*[1], *Loredana Perego*[1], *Nunzia Della Malva*[1], *Riccardo Rubini*[1], *Annarita Lincesso*[1], *Agnese Scatigno*[2], *Erica Rosina*[1], *Luigina Spaccini*[3], *Maria Iascone*[1]

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Background: Shwachman-Diamond syndrome (SDS) is caused by biallelic variants in the SBDS gene, which shares 97% sequence homology with the pseudogene SBDSP1. Both genes are expressed in different tissues. Gene conversion is a known disease mechanism, which may lead to unexpected inheritance and failure to detect pathogenic variants by enrichment-based approaches.

Material and Methods: We performed genome analysis of the proband and parents for family 1 and of two fetuses and parents for family 2 on DNA extracted from peripheral blood or amniocytes. As a validation, we performed RNA sequencing from PAXgene blood samples from family 1 and control samples.

Results: WGS analysis detected in the family 1 the known variants c.258+2T>C on the maternal allele and c.183-184TA>CT and c.201A>G de novo and with a low VAF on the other allele, and c.258+2T>C and c.428C>T in both fetuses of the second family, inherited from the mother and father, respectively. The presence of the pseudogene SBDSP1 can lead to misalignment during genome analysis. Therefore, we performed RNA analysis since the two transcripts present different exon-intron structures, ensuring the exclusive analysis of the SBDS gene. In this way, we characterized the identified variants: the c.258+2T>C causes the production of several aberrant transcripts and the apparently de novo variants c.183-184TA>CT and c.201A>G are due to a postzygotic gene conversion event.

Conclusion: WGS analysis is a practical approach for diagnosing SDS and many other diseases caused by complex genomic rearrangements. In addition, RNA sequencing is a proper complementary method to deepen ambiguous genetic alterations.

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P15.082.A A system for RNA analysis of the *CFTR* splicing variants identified in patients with cystic fibrosis

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Background: Cystic fibrosis (CF) is caused by pathogenic variants in the *CFTR* gene, approximately 10% of which are splicing errors. Understanding the pathogenetic mechanisms of splicing variants is crucial for clarifying gene-phenotype correlations and developing gene therapies.

Material and Methods: Total RNA was isolated from patient-derived samples, followed by RT-PCR analysis. PCR-products were sequenced using various NGS platforms and processed via an in-house pipeline.

Results: To analyze splicing variants in the *CFTR* gene, we developed a system for RNA analysis. Since *CFTR* expression is low in commonly available clinical tissues, such as primary skin fibroblasts or blood, we used intestinal organoids and cultured nasal epithelium for RNA isolation. We developed and tested primers that enable the amplification of the entire *CFTR* coding

region as a single locus from cDNA (4608 bp). Next, we performed NGS of the obtained PCR product, achieving an average coverage of ~100,000× across the *CFTR* CDS. This allowed us to detect minor splicing events (subject to NMD degradation or residual expression of the reference isoform) and the quantification of allele expression disbalances. The system underwent validation using healthy control samples and the known splicing variant as well as the established missense variant. In addition, we analyzed several patient-derived samples stored in the SRC "Biobank" of the Research Centre for Medical Genetics and determined the pathogenic mechanisms of these variants.

Conclusion: This study highlights the complexity of splicing defects and improves our understanding of CF pathogenesis, which is key to the development of new therapeutic strategies.

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P15.083.B Multiple Endocrine Neoplasia and Sporadic Insulinomas

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Background: Functional pancreatic neuroendocrine tumors (PNETs) can be hereditary or sporadic. Hereditary forms are often associated with MEN1 mutations, while most insulinomas are sporadic, solitary, and low-grade. However, the tumorigenesis of sporadic insulinomas remains poorly understood. This study investigates secondary events following the first-hit MEN1 variant and the mutational profiles of both MEN1-associated insulinomas and sporadic tumors.

Materials and Methods: Tissue samples were collected from 4 patients with MEN1 syndrome and 12 patients with sporadic PNETs. Analyses included NGS exome sequencing, transcriptomics, histology, and immunohistochemistry (IHC). Variant calling utilized the GATK pipeline with variant filtering in Varseq software. CNV and LOH analyses were conducted using Varseq, employing Z-scores, ratio plots, and VAF metrics. RNA was extracted and hybridized to Clariom S arrays, with data processed in the Transcriptome Analysis Console (TAC) and refined using R scripts. IHC staining was performed for insulin, glucagon, synaptophysin, islet-1, and Ki67 across all insulinoma specimens.

Results: This study represents the first to evaluate MEN1 second-hit occurrences in PNETs from young MEN1 patients at DNA, RNA, and protein levels. Sporadic insulinomas exhibited significant genetic heterogeneity with few recurrent alterations among candidate genes.

Conclusion: Findings provide novel insights into beta-cell pathophysiology and insulin secretion, offering a framework for future research on therapeutic targets in diabetes and hyperinsulinism. Results were further corroborated through meta-analysis with published exome and genome sequencing data of well-differentiated MEN1 and sporadic PNETs.

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P15.084.C In-depth molecular analysis of *TSC1* and *TSC2* genes in 76 Polish tuberous sclerosis complex probands, with a focus on detecting low-level mosaicism in blood

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Background: Tuberous sclerosis complex (TSC) is a rare tumor predisposition syndrome caused by the inactivation of the *TSC1/TS2* genes, leading to the development of hamartomas in multiple organs. Early diagnosis is crucial for effective clinical management and genetic counseling; however, it is often hindered by the challenge of detecting low-level mosaicism. Here, we demonstrate that implementing duplex sequencing alongside standard diagnostic approaches can increase the sensitivity of *TSC1/TSC2* variant detection, potentially up to 92%.

Material and Methods: We performed targeted next-generation sequencing (NGS), complemented by multiplex ligation-dependent probe amplification (MLPA) analysis, on blood-derived DNA from 76 probands with a definitive clinical diagnosis of TSC according to the current diagnostic criteria. To identify low-level mosaicism, we implemented duplex sequencing, an ultra-sensitive NGS modification that significantly reduces the sequencing error rate, enabling the detection of pathogenic variants at extremely low frequencies.

Results: A total of 85.5% (65/76) likely pathogenic or pathogenic variants were identified in *TSC1/TSC2* using the standard diagnostic approach, including twelve mosaic variants with allele frequencies (AF) ranging from 5.1% to 32.7%. Samples from the remaining 11 individuals, initially classified as negative results, were subjected to duplex sequencing, leading to the detection of *TSC1/TSC2* pathogenic variants in five additional individuals, with the lowest detected AF being 0.3%.

Conclusion: Duplex sequencing emerges as a promising complementary tool that increases the detection rate of previously undiagnosed cases of low-level mosaicism in TSC-related patients. This approach could facilitate TSC diagnosis and enable the timely implementation of appropriate medical surveillance.

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P15.085.D HiFi long-read sequencing of repeat expansions: applications for precision genetics in a clinical setting

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Background: The Unit of Neurogenetics at the Carlo Besta Institute is a major Italian referral center for the clinical and genetic diagnosis of neurodegenerative diseases caused by Repeat Expansions (RE). The aim of this study was to exploit the power of HiFi single-molecule sequencing technology (Pacific Biosciences, PacBio) to elucidate the architecture of complex REs and validate the data obtained by routine laboratory techniques.

Material and Methods: Thirteen subjects previously diagnosed with RE (n = 7 SCA8/*ATXN8OS*, n = 1 FXTAS/*FMR1*, n = 1 FRDA/*FXN*, n = 3 FTDALS1/*C9orf72*, n = 1 CANVAS/*RFC1*) underwent long-read sequencing (LRseq) on a PacBio Sequel IIe sequencer with the PureTarget RE panel kit.

Results: We obtained an average coverage of ~433X in the 20 regions analyzed. RE lengths were concordant with those estimated by PCR, except for a systematic discrepancy of +3(CAG) in *ATXN1* detected by LRseq. Sequencing of *RFC1* RE revealed the complexity of this region, characterizing one carrier of a pathogenic AAGGG expansion and various short alleles with alternate motifs. Sizing and sequencing of the *C9orf72* RE was inefficient in 1 patient, likely due to the RE length causing allele drop-out. Moreover, PacBio sequencing allowed to distinguish between CTA and CTG tracts in the challenging *ATXN8OS*/SCA8 RE, thus making it possible to size exclusively and precisely the disease-causing CTG repeat.

Conclusion: HiFi PacBio sequencing allows to improve definition of RE, contributing to reduce systematic errors of fragment analysis and to elucidate the complex architecture of critical REs. Extension of the study to a larger number of patients and conditions is ongoing.

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P15.086.A Relative gene expression and translation efficiency of *CALM1*, *CALM2*, and *CALM3* may contribute to variation in severity of calmodulinopathy

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Background: Ultra-rare missense variants in the *CALM1*, *CALM2*, and *CALM3* genes, which encode identical calmodulin proteins, can cause calmodulinopathy, a spectrum of various clinical manifestations, including ventricular arrhythmias and sudden cardiac death. However, disease-causing variants are not observed with equal frequency among the three calmodulin encoding genes in clinical studies. This observation may be explained by distinct roles of the three genes. Consequently, the phenotypic severity may depend on which of the calmodulin encoding genes that is affected by a missense variant.

Materials and Methods: We used data from cardiac tissues of 466 individuals to compare the gene expression levels and translation efficiency of *CALM1*, *CALM2*, and *CALM3*.

Results: In the human heart, *CALM2* displayed the highest expression level with a median expression level 15.2% higher than *CALM1* and 90.1% higher than *CALM3*. Furthermore, the translation efficiency of *CALM1* and *CALM2* was 139.5% and 105.4% higher than that of *CALM3*.

Conclusion: These differences in expression levels and translation efficiencies indicate that missense variants in *CALM3* may be less severe than those in *CALM1* and *CALM2*. Consequently, gene-specific variant interpretation may be required in the clinical management of calmodulinopathy.

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P15.087.B Advancing Genome Sequencing for Neurodevelopmental Disorders: integrating structural variant calling, classification, and prioritization

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Background: GS is increasingly used as a first-line diagnostic test for patients with rare genetic diseases such as neurodevelopmental disorders (NDDs). Its clinical applications require a robust infrastructure for processing, storage and analysis. Moreover, Structural Variants detection and interpretation remain challenging, and a prioritization system is needed to facilitate downstream clinical analysis. Here, we present the results of a clinical application of WGS in a cohort of 54 families with NDDs.

Methods: We developed portable workflows for GS data-processing and SNV/SV/STR calling. A benchmark of state-of-art SV detection tools identified the most accurate combination for SV calling, and a machine-learning model was trained to assess reliability of SV detection combining calls from different state-of-art tools. Additionally, a gene-based prioritization system was implemented to support variant interpretation.

Results: Our benchmark analysis optimized SV detection by selecting the most accurate tools combination and integrating them into a dedicated pipeline. An xGBoost was trained (within a 10-fold cross-validation, repeated 10 times) to assess SV reliability, achieving robust AUROC on test set (0.869 ± 0.014). The prioritization system supported downstream analysis leading to a molecular diagnosis in 21.8% of patients, with SV accounting for 16%. Additionally, it suggested a potential diagnosis in up to 14.5% of cases, mainly consisting of non-coding variants.

Conclusion: Our findings highlight that integrating SNV/SV analysis significantly enhances the diagnostic yield of GS, although potential finding may need additional layers of analysis to be thoroughly interpreted. Moreover, implementing a dedicated pipeline improves variant detection, classification and interpretation, supporting more accurate molecular diagnoses.

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P15.088.C Challenges in prioritising disease-causing variants in recessive monogenic diseases observed in homozygous state in population databases

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Background: Allele frequencies from population databases are crucial for prioritizing and interpreting rare variants associated with Mendelian disorders in genomic analysis.

Material and Methods: A retrospective analysis was conducted on data from individuals with a molecular diagnosis at our centre to identify probands carrying variants associated with rare genetic disorders, observed in a homozygous state in the gnomAD (v4.1.0) population database.

Results: We herein report five individuals from four unrelated families with three distinct autosomal recessive disorders. In family 1, we identified, c.193G>T p.(Gly65Trp) and c.956A>G p.(Tyr319Cys) in compound heterozygous state in GALC (NM_000153.4), causing later-onset Krabbe disease (MIM #245200). The variant c.956A>G p.(Tyr319Cys) is reported in 14 individuals in a homozygous state in gnomAD (Allele frequency:0.0007902). In family 2, we identified c.275-2A>G and c.680G>A p.(Arg227Lys) in compound heterozygous state in MECR (NM_016011.5) causing optic atrophy 16 (MIM #620629) in two similarly affected siblings. The variant c.680G>A is reported in 12 individuals in a homozygous state (Allele frequency:0.001979). In family 3 and family 4, c.2179G>A p.(Val727Met) in compound heterozygous state with c.1505G>A p.(Gly502Asp) and c.1664C>T p.(Ala555Val) were noted, respectively in GNE (NM_001128227.3), causing Nonaka myopathy (MIM #605820). The variant c.2179G>A is reported in 12 individuals in a homozygous state (Allele frequency :0.0007453).

Conclusion: The likely explanations for the presence of disease-causing variants for recessive monogenic diseases in population databases include possible hypomorphic variants, late-onset disorders, and non-penetrance. An integrated clinical and genomic approach to variant interpretation is likely to result in improved identification of these variants in genomic testing pipelines.

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P15.089.D Population prevalence of postzygotic variants at preclinical frequency in the *UBA1* gene

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Background: Increasing evidence supports that somatic variants contribute to non-cancer diseases, mostly monogenic but also complex disorders. Notably, gene mosaicism plays a relevant role in the pathogenesis of inborn errors of immunity, especially in the group of autoinflammatory diseases (AIDs). Clinical manifestations in certain AIDs appear later in life, suggesting the expansion of preexisting mutant clones through clonal hematopoiesis. Herein we aim to characterize the mutational load of somatic variants at subclinical frequencies in *UBA1* causing the VEXAS syndrome and other AIDs genes.

Material and Methods: We have analyzed whole-genome sequences from 490,000 individuals aged 40–70 years in the UK Biobank repository. A bioinformatic pipeline with GATK and Variant Effect Predictor was applied to detect low-frequency variants, combined with a set of filters based on mapping quality, minimum reads supporting the alternative allele, strand bias and proximity to read ends and indels, was applied to reduce false positives.

Results: We have identified carriers of pathogenic variants in *UBA1* and *NLRP3*, some of them previously reported as pathogenic in AIDs. These results have enabled to establish a characterization of the load of somatic variants in these genes for males and females, as well as performing a spatial distribution analysis of the germline and somatic variants.

Conclusion: The genetic load of somatic variants in unaffected individuals is informative on the number of individuals at risk of developing an AID of postzygotic origin, which can be of relevance for physicians visiting patients with late-onset inflammatory disorders.

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P15.091.B Deciphering the molecular signature in Kleefstra syndrome

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Background: Kleefstra syndrome (KLEFS, OMIM #610253) is a rare genetic disorder, characterized by moderate-to-severe intellectual disability, hypotonia and distinctive gestalt, variably associated to seizures and congenital defects. Causative alterations, both single nucleotide- or copy number- variants, affect *EHMT1*, an euchromatin histone methyltransferase.

Materials and methods: This project develops on three synergic paths. *Clinical study* Clinical reports have been collected, reviewed and compared to literature, in order to better define study population and build a dedicated registry. *Molecular study* All molecular tests were revised to define population genotypes and to correctly label individuals harbouring *EHMT1* uncertain

variants. In vitro *study* Starting from patients' blood cells, personalized in vitro models were created, aiming to investigate etiopathological processes and to test novel therapeutic approaches.

Results: Exhaustive clinical and genetic data were gathered for 38 patients, including pregnancy history, auxological parameters, cardiological and neuropsychiatric evaluations. All patients were also re-evaluated from a gestaltic point of view.The cohort phenotypical and mutational repertoire will be described generating a comprehensive overview of this rare condition. The results of genome wide scanning on mislabelled cases will help to acquire a critical viewpoint when facing a variant in *EHMT1*. In collaboration with the Gaslini Telethon Genetic Bank service, we generated 36 lymphoblastoid cell lines. Preliminary results of in vitro studies will be presented.

Conclusion: This innovative holistic bench to clinic study on KLEFS provides a deeper knowledge of this rare disorder, of its genotypic and phenotypic landscape with the final aim to develop targeted therapies.

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P15.092.C Clinical Application of Whole Genome Sequencing in a Russian Cohort: Insights from 3600 Genomes

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Background: Whole genome sequencing (WGS) is the most comprehensive molecular diagnostic tool, yet its clinical adoption remains limited despite declining costs outpacing whole exome (WES) and panel sequencing. While WGS is predominantly utilized in research and population studies, it is rarely first-line in clinical practice due to persistent cost and infrastructure barriers. This study evaluates the diagnostic utility of WGS in a large Russian cohort with unresolved genetic conditions.

Material and Methods: WGS was performed on 3,646 DNA samples (patients and relatives) using PCR-free enzymatic fragmentation (MGI) and the DNBSEQ-T7 platform (paired-end 150 bp reads). Collaborative efforts under the national "100,000+ Me" initiative enabled sequencing and analysis of 1,876 families over one year. Trio/quadro analysis comprised 50% of cases.

Results: Causative/likely pathogenic (P/LP) variants were identified in 34% of cases (2,088 unique variants; 65% novel). Despite prior targeted testing, WGS provided an 8.2% incremental diagnostic yield. Variant distribution included SNPs (85%), deep intronic (11%), CNVs (3%), and rare mitochondrial/STR/structural variants (common <1%). The diagnostic yield varied across disease groups, ranging from 18% for epilepsy to 67% for Rubinstein-Taybi syndrome. The cohort exhibited selection bias, as part patients underwent prior panel/WES testing per diagnostic protocols.

Conclusion: While WGS incurs costs beyond sequencing (data storage, infrastructure, security), its clinical adoption is rising, particularly for ultra-rare disorders. This study underscores WGS's unique capacity to resolve complex cases, urging a paradigm shift in diagnostic workflows for rare diseases.

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P15.094.A Integrating phenotype data and de novo filtering to prioritise non-coding variants in clinical whole genome sequencing

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Background: Whole genome sequencing (WGS) has become the first-line diagnostic test for severe paediatric rare diseases in the NHS England Genomic Medicine Service (NHSE GMS). However, current analysis is limited to coding regions, due to challenges in prioritising and interpreting non-coding variants. Increasing evidence for the role of non-coding variation in rare disease underscores the need for effective analysis strategies. This study examines whether filtering de novo single nucleotide variants (SNVs), in genes from the NHSE GMS R27 Paediatric Disorders expertly curated gene panel, can effectively identify clinically relevant non-coding variants.

Material and Methods: Variant call format (VCF) files were obtained from local GMS cases with no causative variants reported. Filtering criteria included de novo SNVs in autosomal dominant or X-linked R27 "green" genes and absence from gnomAD. Variants with a coding consequence, autosomal recessive inheritance and all indel variants were excluded. Variants were annotated using Ensembl Variant Effect Predictor v113.0 with gnomAD v4.1 and SpliceAI delta scores.

Results: A total of 172 cases were analysed, yielding 484 autosomal dominant de novo non-coding variants. Of these, 246 variants across 116 cases were determined to be potentially phenotypically relevant. Stringent assessment of patient HPOs narrowed this down to 16 variants for further analysis. Full results will be presented at the conference.

Conclusion: This approach demonstrates the feasibility of expanding current WGS analysis to include non-coding regions. Robust filtering and prioritisation strategies are required to incorporate phenotype and in silico prediction tools to allow identification of clinically relevant variants.

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P15.095.B Regional Clustering of the COL7A1 c.5047C>T Variant in Recessive Dystrophic Epidermolysis Bullosa Patients in Brazil

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Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited skin disorder characterized by recurrent blistering beneath the cutaneous basement membrane, leading to scarring, contractures, and gastrointestinal strictures. Diagnosis is confirmed by clinical findings and biallelic pathogenic variants in the COL7A1 gene. Emerging therapies, such as COL7A1-targeted gene therapy, show promise. Guide et al. (2022) demonstrated significant wound healing in RDEB patients treated with topical B-VEC (beremagene geperpavec), a herpes simplex virus-based gene therapy.

Material and Methods: Brazil's diverse, admixed population provides a unique setting to evaluate COL7A1 variants. We analyzed whole-genome sequencing data from the BRGP (Brazilian Rare Genomes Project), a PROADI-SUS consortium with the Israelita Albert Einstein Hospital, encompassing 7,375 patients with rare diseases across 28 centers.

Results: Among the 7,375 probands analyzed, 37 patients (0.50%) were found to carry pathogenic bi-allelic variants in the COL7A1 gene. Of these, 18 (48.6%) harbored the loss-of-function variant c.5047C>T; p.(Arg1683Ter). Within this group, 4 patients (22.2%) were compound heterozygous, and 14 (77.8%) were homozygous. Remarkably, all 18 patients originated from Bahia State, indicating a potential founder effect.

Conclusion: Our findings reveal a significant clustering of RDEB patients in Brazil, particularly in Bahia linked to the c.5047C>T variant in COL7A1. This regional concentration highlights the importance of targeted diagnostic and therapeutic strategies, including emerging treatments like B-VEC, and tailored genetic counseling to address the unique genetic landscape and healthcare needs of this population.

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P15.097.D A novel GPR143 mutation in a large Iranian family with X-linked nystagmus

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Background: Congenital Nystagmus syndrome is a genetic disorder characterized by involuntary oscillation of the eyes starting within the first six months of life. While many genes associated with this condition have been identified, the objective of this study is to discover a new mutation contributing to this syndrome.

Material and Methods: This family-based study investigated a 4-generation Iranian family with nystagmus. Whole exome sequencing, Sanger sequencing for mutation confirmation, segregation analysis, bioinformatics tools, and in silico protein modeling were utilized.

Results: A rare hemizygous missense variant in the G-protein coupled receptor 143 (GPR143) gene was identified in affected males. The mothers of the affected individuals were heterozygous carriers, and unaffected males did not carry the mutation. Protein modeling revealed that the p.Ala2Asp mutation is located near the Beta-1 adrenergic receptor interaction site, likely causing structural instability.

Conclusion: This study identifies a novel X-linked recessive missense mutation in the GPR143 gene associated with congenital nystagmus. These findings may have implications for genetic studies in the Iranian population and beyond.

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P15.098.A Genetic analysis of a national biliary atresia cohort reveals high heterogeneity in causative genes

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Background: Biliary atresia (BA) is a rare, rapidly progressive cholangiopathy exclusive to newborns, characterized by fibro-obliterative destruction of the bile ducts. As one of the most common causes of neonatal cholestasis and treatable through timely surgical intervention, BA requires prompt diagnostic evaluation. The first-line surgical treatment, Kasai portoenterostomy (PE), aims to restore bile flow and prolong survival with the native liver. However, despite PE, 70–80% of BA patients eventually require liver transplantation (LT) before adulthood, making them the largest group (40–50%) of pediatric LT recipients worldwide. The advent of genetic testing has transformed the evaluation of neonatal cholestasis, providing opportunities for accurate molecular diagnoses. Despite this, only a limited number of BA-linked genetic variants have been identified to date.

Material and Methods: Whole-exome sequencing (WES) was conducted to identify rare genetic single nucleotide and copy number variants, while genome-wide association study (GWAS) analysis was performed to investigate common SNP associations across the cohort of 48 BA patients. To further validate the functional impact of selected candidate variants, immunostaining and flow cytometry were performed on patients' skin biopsy or PBMC.

Results: While GWAS did not reveal significant SNP associations, WES identified plausible rare candidate variants in 11 patients, including a copy number gain in chromosome 22 in one case. However, for the majority (77%) of patients, no plausible rare disease-causing variants were detected.

Conclusion: Our findings highlight the genetic complexity of biliary atresia, supporting the hypothesis that BA is primarily a multifactorial disease.

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P15.099.B Robust and accurate genome analysis with SOPHiA DDM™ WGS solutions

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SOPHiA GENETICS SA, Rolle, Switzerland.

Background: Whole-genome sequencing (WGS) provides unparalleled insights into the genetic basis of rare diseases. To fully leverage WGS, accurate, scalable, and comprehensive genomic analytic solutions are crucial.

Material and Methods: We developed two WGS solutions integrating proprietary algorithms with public tools for variant detection, annotation, and reporting; SOPHiA DDM™ low-pass WGS (lpWGS) solution offers an alternative to chromosomal microarray analysis (CMA) for CNV and RoH detection, while the 30xWGS solution analyses all variant types. Their performance was evaluated using the Genome in a Bottle (GIAB) and 1000 Genomes Project (1KGP) reference materials, and simulated data. The lpWGS solution was compared to CMA, while 30xWGS was benchmarked against leading solutions.

Results: The 30xWGS solution achieved >99% sensitivity, precision, and F1-score for SNV/indel detection. Mitochondrial variant calling reached >97% accuracy with a heteroplasmy threshold of 5%. Structural variant detection showed 75% sensitivity and 94% precision, outperforming other leading SV callers. CNV benchmarking achieved 94% recall and 77% precision, surpassing established CNV detection tools. Analysis of simulated CNV data demonstrated 95% sensitivity for detecting constitutional CNVs ≥10 kb and mosaic CNVs ≥5 Mbp at 30% mosaic fraction. The lpWGS solution achieved >95% sensitivity for constitutional CNVs ≥30 kb and mosaic CNVs ≥5 Mbp at 30% mosaic fraction. RoH detection with both pipelines showed >99% sensitivity for events ≥5 Mbp.

Conclusion: The SOPHiA DDM™ lpWGS and 30xWGS solutions demonstrated high sensitivity and precision across all variant types surpassing established gold standards. Their robust performance and scalability make them powerful tools for comprehensive genomic analysis.

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P15.100.C SH2D1A-Related X-Linked Lymphoproliferative Disease goes beyond Epstein-Barr Virus: Varicella-Zoster as an Atypical Trigger

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Background: The SH2D1A gene encodes the SLAM-associated protein (SAP), a key regulator of T-cell signaling critical for effective immune responses. Mutations in SH2D1A are the primary cause of X-linked lymphoproliferative disease (XLP), a rare immunodeficiency marked by abnormal responses to Epstein-Barr virus (EBV), autoimmune manifestations, lymphomas, and internal bleeding complications due to herpes family virus infections. Understanding the genetic basis of such conditions is essential for early intervention and family counseling.

Material and Methods: Two male patients, aged 11 and 19, were evaluated for immune dysregulation. Whole Exome Sequencing (WES) identified pathogenic variants in SH2D1A. Parental testing via Sanger sequencing clarified inheritance patterns. The clinical data was analyzed to help correlate genotype with phenotype.

Results: The 11-year-old patient presented with eosinophilic colitis, chronic mucositis, dermatitis, pneumonitis with granulomatous lesions, and intermittent fever. A c.137+5G>A variant in SH2D1A was detected, leading to a 22 bp deletion in exon 1 and loss of SAP function. This maternally inherited variant disrupts splicing, emphasizing the need for genetic counseling. The second patient, a 19-year-old male, exhibited multiple aneurysms and iron deficiency anemia. WES revealed a maternally inherited c.313_315del, p.(Lys105del) variant, previously associated with thrombotic vasculopathy and aneurysms in EBV-infected patients. While EBV is the typical trigger in SH2D1A-related immune dysregulation, this patient's disease was initiated by herpes zoster from varicella-zoster virus (VZV). Notably, the spleen and kidney aneurysms in the reported case required surgical removal due to hemorrhage.

Conclusion: SH2D1A mutations lead to severe immune dysregulation and varied systemic complications without clear targeted therapies.

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P15.101.D Combining bio-informatic tools to facilitate implementation of non-coding variant analysis in genome diagnostics

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Background: Diagnostic yield for solving rare genetic diseases remains around 30–40%. Current analyses are focused on variants of the coding genome and ignore most non-coding (nc) variants in important regulatory regions. Our aim is to develop a diagnostic-proof filter strategy for non-coding variant analysis, to augment current NGS testing.

Material and Methods: WGS data from 1 positive case (known deep-intronic splicing variant) and 10 unsolved cases, previously analyzed for neurodevelopmental phenotypes, were examined. Two filter strategy tools (VariantInterpretationPipeline (VIP, in-house) and GREEN-VARAN (G-V, open-source)) were used to identify variants in promoter/enhancer, intronic and UTR regions

using FATHMM-MKL, ReMM and ncER algorithms. In VIP, SpliceAI and UTRAnnotator algorithms were used to identify splice and 5'UTR variants. Finally, filtering for 5045 (mainly) neurodevelopmental associated genes was applied. The two filter strategies were compared and clinical review was performed.

Results: The two filter strategies resulted in varying variants of interest (VOI): VIP = 1321 ± 323 and G-V = 138 ± 46. Application of gene panel filtering reduced the number of VOIs to 21–51 for VIP, and 4–14 for G-V. The VIP/G-V overlap (including splice and 5'UTR variants) reduced the number of VOIs to 5–17, and clinical review led to 0–3 variants per individual. Notably, the positive control splice variant was prioritized in VIP. Follow-up includes parental inheritance control and functional testing: RNAseq to confirm spicing of VOI in *TLK2*, and methylation pattern testing for *ZEB2* enhancer VOI.

Conclusion: Our results demonstrate that applying a diagnostic-aimed filter strategy incorporating existing bio-informatic tools makes diagnostic non-coding variant analysis feasible.

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P15.102.A Whole Genome Sequencing-based Identification of a Novel Frameshift Mutation in the *STK11* Gene in Peutz-Jeghers Syndrome

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Background: Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant disorder characterized by gastrointestinal polyposis and mucocutaneous pigmentation, typically caused by *STK11* mutations. However, some patients exhibit PJS features without these mutations. Here, we report the case of a 30-year-old man with suspected PJS, partial symptoms, and an unknown genetic background. He and his asymptomatic parents participated in a study aimed at creating the Genomic Map of Poland.

Material and Methods: Whole genomes were sequenced at 30x coverage using DNBSEQ (MGI platform). To identify potentially pathogenic variants, we analyzed SNPs and indels using five approaches: i) *STK11* gene only, ii) HIGH-impact variants determined by Variant Effect Predictor (Ensembl), iii) variants from ClinVar database, iv) COSMIC database variants, and v) pathogenic and likely pathogenic variants determined by eVai (enGenome).

Results: Based on the whole genome sequencing (WGS), we identified a heterozygous frameshift mutation in the *STK11* gene, not reported previously. This variant is a single nucleotide deletion (c.171delA) that alters the amino acid sequence in the N-terminal part of the protein (p.Gly58fs). The mutation was found exclusively in the patient and absent in both parents, indicating de novo origin. WGS results were confirmed by Sanger sequencing of the affected *STK11* gene fragment.

Conclusions: Our findings, combined with medical examinations, strongly suggest that the identified novel frameshift mutation in *STK11* may be responsible for the patient's phenotype. This case underscores the importance of comprehensive genetic analysis in the diagnosis of PJS and other rare diseases, particularly in patients lacking typical disease-related phenotypes.

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P15.103.B Multi-Omics Approach Reveals the Impact of Prognosis Model-Related Genes on the Tumor Microenvironment in Medulloblastoma

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Background: The tumor microenvironment (TME) significantly impacts the progression and prognosis of medulloblastoma (MB). This study aimed to develop a TME-associated risk score(TMErisk) model using RNA sequencing data to predict patient outcomes and elucidate biological mechanisms.

Material and Methods: RNA sequencing data from 322 Tiantan and 763 GSE85217 MB samples were analyzed. Key gene modules related to immune and stromal components were identified using Weighted Gene Co-expression Network Analysis (WGCNA). Significant genes were screened using LASSO-COX and COX regression models. Single-cell RNA sequencing (scRNA-seq), single-cell ATAC sequencing (scATAC-seq), and spatial RNA analyses validated the findings.

Results: Differential expression analysis identified 731 upregulated and 15 downregulated genes in high vs. low immune score MB patients, and 686 upregulated and 43 downregulated genes in high vs. low stromal score patients. Eight key genes (*CEBPB*, *OLFML2B*, *GGTA1*, *GZMA*, *TCIM*, *OLFML3*, *NAT1*, and *CD1C*) were included in the TMErisk model, which demonstrated strong prognostic power. High TMErisk scores correlated with poorer survival, distinct immune cell infiltration patterns, and lower tumor cell stemness. Single-cell analyses revealed the expression dynamics of TMErisk genes across cell types, including macrophages, T cells, and NK cells, and identified key regulatory transcription factors. Spatial transcriptomics showed significant clustering of TMErisk genes in tumor regions, highlighting spatial heterogeneity and the formation of immune hubs.

Conclusion: The TMErisk model enhances our understanding of the MB tumor microenvironment, serving as a robust prognostic tool and suggesting new avenues for targeted therapy.

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P15.105.D Polygenic Risk Scores (PRS) and Pharmacogenetic Card Profiling Analysis in a Cohort of 35 Patients with Wolff-Hirschhorn Disease

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Background: Wolf-Hirschhorn syndrome (WHS) is a rare genetic disorder characterized by a deletion of the distal short arm of chromosome 4p16.3. Clinically, WHS presents with developmental delay, intellectual disability, distinctive craniofacial features, and seizures. Interestingly, WHS shows a great variability in the epilepsy management among patients. Despite advances in genetic characterization, the role of polygenic influences and pharmacogenetics in WHS remains underexplored.

Materials and Methods: We analyzed polygenic risk scores (PRS) for 130 traits in 35 WHS patients to investigate potential correlations with phenotype, by using WGS. PRS values were extracted, and traits with percentile scores above 90 and 95 were examined systematically. Additionally, pharmacogenetic profiling for 49 medications was

performed using PharmCat, focusing on gene-drug interactions and clinical recommendations. Data were categorized by patient with a specific emphasis on antiepileptic drugs.

Results: The traits "Insomnia," "COVID susceptibility" "Irritable bowel syndrome" and "periodontitis" exhibited PRS values exceeding the 90th percentile suggesting potential commonalities in polygenic influences within this cohort. Pharmacogenetic analysis revealed that 28 patients (and nine unique genes) had specific recommendations for at least one of the 49 medications studied. However, no recommendations for antiepileptic drugs were identified. The results highlight variability in pharmacogenetic profiles within the cohort, emphasizing the need for personalized medicine approaches.

Conclusion: This study provides a comprehensive overview of polygenic and pharmacogenetic influences in WHS. The pharmacogenetic analysis further emphasizes the clinical relevance of genetic profiling in optimizing therapeutic strategies. Future research should aim to explore the integration of PRS and pharmacogenetics in patient care.

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P15.106.A The Clinical Impact of DNA Methylation Episignatures Testing: Insights from a Multicenter Cohort Study in Portugal

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Background: Methylation arrays are a high-throughput technique for evaluating unique DNA methylation patterns, known as episignatures. An increasing number of rare disorders have been linked to specific episignatures, providing quick and accurate diagnoses across a wide range of clinical applications.

Material and Methods: This retrospective study analyzes the first group of Portuguese patients who underwent DNA methylation testing from January 2023 to June 2024 using Illumina Infinium Methylation EPIC BeadChip arrays and the EpiSign assay. Testing was conducted by national Medical Genetics Departments, which defined the criteria. The study included two groups: 1 - subjects with variants of unknown clinical significance in genes with known episignature and clinical overlap; 2 - subjects with inconclusive genetic results but a diagnosis aligning with syndromes that have established episignatures.

Results: In our cohort of 48 cases, 44% showed positive results for episignature or imprinting changes, with highly reliable results. In Group 1, 50% of cases tested positive for a episignature compatible with the previously identified genetic variant, allowing us to label it as likely pathogenic and achieve a clear diagnosis. In Group 2, 41% of patients had an episignature that confirmed clinical diagnosis, strengthened it and guided individual recommendations for further testing.

Conclusion: Our study found a high rate of positive results, likely due to careful patient selection. A combined clinical and laboratory approach was crucial for accuracy. We believe episignature testing is useful for clinical practice and that genetics departments in Portugal are ready to use it regularly and expand its applications.

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P15.107.B Uncovering population-specific pathogenic variants in recessive monogenic diseases: insights from North Macedonia

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Background: Massive parallel sequencing (MPS) has enabled comprehensive testing for carrier status in recessive monogenic diseases (RMDs) and has revealed that variant frequency differences necessitate population-specific adjustments in RMD carrier screening. Our aim was to identify population-specific pathogenic variants in RMDs from MPS data in North Macedonia.

Materials and Methods: We analyzed exome sequencing data from 1,269 patients referred to our laboratory for genetic testing for rare diseases. We searched for ClinVar pathogenic variants (version 06-Jan-2025) in 1,283 genes linked to childhood-onset RMDs (panelapp.agha.umccr.org/panels/3139). Filtering criteria included variant quality, allele frequencies, and ClinVar review status. Alleles causing the patients' clinical phenotypes were excluded from the count.

Results: A total of 2,086 alleles, including 918 distinct pathogenic variants in 482 genes, were identified, of which 92 variants (679 alleles in 86 genes) showed significantly higher allele frequencies compared to gnomAD exomes v.2.1 after applying Bonferroni correction, and were designated as population-specific variants. As expected, cystic fibrosis and phenylketonuria were among the top-ranked genes with the highest gene carrier frequencies. However, similar allele frequencies were observed for other genes/diseases, revealing a discrepancy between the expected and observed disease incidence in North Macedonia (internal data). This discrepancy may result from prenatal lethality associated with highly deleterious variants (e.g., CPLANE1:c.1819-del;7871T>A, DHCR7:c.452G>A, DDX11:c.1403dup) or mild

phenotypes due to hypomorphic variants (e.g., MKS1:c.1476T>G), leading to potential underdiagnosis.

Conclusion: MPS from genetic testing can be further utilized to inform national health policies by highlighting potential clinical underdiagnosis and guiding the design of preconception carrier screening programs.

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P15.108.C DNA methylation analysis for diagnostic testing in neurodevelopmental disorders

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Background: DNA-methylation analysis is increasingly playing a crucial role in diagnosing neurodevelopmental disorders(NDDs) by identifying specific episignatures, now associated with over 90 conditions. This study aimed to evaluate DNA-methylation patterns in NDD patients as part of a multicentric project.

Material and Methods: A subset of 16 deeply phenotyped cases was examined, which previously tested negative for fragile-X-syndrome, chromosomal microarray and trio-exome sequencing. Methylation profiling was performed with Infinium-MethylationEPIC v2.0 BeadChips and data were analyzed using the Episign v4.0 platform.

Results: We detected known episignatures in six out of 16 patients. A *SETD5*-associated pattern for Mental Retardation, Autosomal Dominant 23 was found in a child with severe growth delay. However, the causative variant was also present in the mildly symptomatic mother but with an uncertain associated methylation pattern. A second pathological condition was suggested in a mother-child pair correlated to the *SMARCA2*:c.3313C>G,p.(Arg1105Gly) variant. The proband showed developmental delay and seizure while the mother was milder. Identified conditions also included Kleefstra-syndrome-1 in one of two clinically similar siblings, later confirmed by the de novo *EHMT1*:c.1384_1385del,p.(Lys462ValfsTer7) variant. Sotos-syndrome was diagnosed in a child with a paternally inherited *NSD1*:c.3004_3005del,p.(K1002EfsTer8) variant. Nicolaides-Baraitser-syndrome was suggested in another case with subsequent identification of causative variant already present in the mother in a mosaic state. Lastly, a patient initially suspected of Cornelia de Lange syndrome(CdLS) exhibited a KBG-syndrome methylation pattern, but no apparent variant was found in CdLS genes.

Conclusion: Our findings underscore the diagnostic value of episignatures, particularly when conventional genetic tests yield inconclusive results.

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P15.109.D Identification of the molecular mechanism underlying the involvement of mutations in the MYO5B gene and cholestasis

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Background: Progressive Familial Intrahepatic Cholestasis (PFIC) comprises rare genetic disorders presenting in infancy with severe itching, hepatosplenomegaly, steatorrhea, and liver failure. While MYO5B mutations have been recently identified in PFIC patients, their pathogenic mechanism remains controversial. Previous studies suggested that mutated MYO5B protein disrupts canalicular protein localization, leading to altered bile salt secretion and cholestasis.

Material and Methods: We analyzed six PFIC patients with MYO5B mutations through genetic analysis and performed protein expression assays on available liver biopsies to investigate canalicular protein localization.

Results: Immunohistochemical analysis of liver biopsies from two patients revealed normal BSEP expression and localization at the canalicular membrane, contrasting with previous hypotheses about MYO5B's role in protein trafficking.

Conclusion: Our findings, along with other unpublished studies, demonstrate preserved BSEP localization in MYO5B-related cholestasis, challenging current understanding of its pathogenic mechanism. Further investigation of MYO5B in cholestasis is crucial to for developing targeted therapies. In vitro models, including induced hepatocytes 2D cells culture and organoids, offer promising platforms to study canalicular protein trafficking and understand the cholestatic phenotype associated with MYO5B mutations.

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P15.110.A An integrated approach to analyze RNA-seq data in the context of rare disorders

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Background: Multi-omics data provide complementary insights that improve the diagnosis of rare genetic disorders. Experience from several international projects has shown that integration of all results into a unified view significantly accelerates interpretation by clinical geneticists.

Materials and Methods: We observed different approaches to analysing RNA-seq alongside DNA data in many projects, including different approaches to reporting RNA results to

researchers interpreting the data, ranging from plotting capabilities using dedicated software to per-family reports in Solve-RD. Analysis of the requirements from these projects led to the development of novel best practices and a user interface.

Results: Here we present our best practices for joint analysis of multiple omics datasets and RNAvision, a novel platform for reporting results. Our pipeline detects approximately 10 outlier genes per sample, significantly reducing the number of dubious findings compared to DROP. The novel user interface includes a summary table of all outlier genes and a common visualisation of all findings and their position within the gene, including variants.

Conclusion: Multi-omics visualisation allows geneticists to seamlessly interpret whole transcriptome data and integrate it into the analysis of rare disease cases. This pipeline can accelerate the adoption of RNA-seq as a complementary assay in human genetics.

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P15.111.B Advancing splicing variant analysis in neurological disorders with targeted long-read RNA sequencing

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Backgrond: Short-read whole-genome sequencing (srWGS) has increased the identification of non-coding variants, underscoring their role in hereditary disorders. Yet interpreting these variants without functional validation is difficult. Amplicon-based long-read RNA sequencing (Amp-lrRNAseq) provides a cost-effective approach to analyze splicing in full-length transcripts while retaining phasing. This study evaluated Amp-lrRNAseq in predicting splicing consequences of non-coding variants.

Material and Methods: Variants in five patients with hereditary neurological disorders were identified by srWGS and prioritized by phenotype and in silico splicing predictions. Amp-lrRNAseq was performed on fibroblasts or whole blood. Quantitative PCR (qPCR) was used to assess variant impact on gene expression.

Results: In a patient with CMT2, we identified a homozygous *GDAP1* variant (c.311-23A>G), previously reported. We confirmed the mutation activates a cryptic splice site, causing the inclusion of intronic nucleotides into exon 2. We also validate previous observations of aberrant splicing caused by a known heterozygous *POLR3A* mutation (c.1909+22G>A) in a spastic ataxia patient. In a patient with Behr syndrome, a novel homozygous deletion in *OPA1* (c.4_5+2del) resulted in skipping of the final exon, with reduced *OPA1* expression in patient fibroblasts. A patient with early-onset myopathy carried a biallelic *PYROXD1* variant (c.85-5A>G) that promotes inclusion of four intronic nucleotides, likely introducing a premature stop codon (p.Gln29fs). Lastly, in an HSP patient, a novel *SPG11* variant (c.2068-498T>G) caused exonization of intron 11.

Conclusion: Amp-lrRNAseq clarifies how genetic variants disrupt splicing and lead to disease, offering a cost-effective, versatile option for variants that match the clinical phenotype but lack definitive pathogenicity evidence.

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P15.112.C Implementation of rapid whole-genome sequencing in neonatal and pediatric ICUs in Czechia: first insights from the nationwide "Baby Fox" study

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Background: Genetic diseases are a leading cause of mortality among neonates and pediatric patients in intensive care units (ICUs). Rapid whole genome sequencing (rWGS) has the potential to revolutionize the diagnosis and treatment of rare diseases, advancing precision medicine in ICU settings. Baby Fox is a prospective multicenter study conducted in neonatal and pediatric ICUs of four tertiary hospitals in Czechia, evaluating the feasibility, diagnostic yield, and clinical utility of rWGS. Here, we present the first findings three months after the study's initiation.

Material and Methods: Patients suspected of monogenic diseases were enrolled following multidisciplinary case discussions. Trio rWGS with phenotype-driven analysis was performed. Demographic data, Human Phenotype Ontology (HPO) terms, rWGS turnaround time, sequencing results, and their actionability (C-GUIDE^{TM}) were analyzed.

Results: Between October and December 2024, 6 newborns (median age 22 days [range 1–90]) and 7 pediatric patients (median age 8 years [range 1–17]) underwent trio rWGS. The median turnaround time was 6 days (range 4–7), with a median of 11 HPO terms per patient (range 4–23). Disease-causing variants, including one non-coding and one structural variant, were identified in 7 patients (3 newborns, 4 pediatric), yielding a diagnostic rate of 54%. Clinical management was modified in 4 patients with molecular diagnoses (57%), including changes in medication and referral to relevant ERN centers.

Conclusion: Despite the project's multicenter nature and logistical challenges, these preliminary findings demonstrate the feasibility of a 7-day turnaround time and expected diagnostic yield, substantiating the clinical utility of rWGS in critically ill children.

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P15.113.D Deciphering the pathogenicity of missense variants in the giant protein Nesprin-1

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Background: Predicting the pathogenicity of missense genetic variants poses a significant challenge, especially in undiagnosed rare diseases. This report describes a patient with early-onset spinocerebellar ataxia carrying two recessive missense variants in the *SYNE1* gene, which encodes Nesprin-1, the second-largest known protein. Initial predictions deemed these variants were benign and VUS, leading to the dismissal of *SYNE1* as a potential candidate. Nevertheless, we experimentally demonstrated a dramatic impact on the Nesprin-1 and the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex function.

Material and Methods: Gene expression studies were conducted using RT-PCR, Western blotting, and immunostaining. Additionally, reverse phenotyping for *SYNE1* was performed in a cohort of 6,189 pediatric patients from our hospital.

Results: Functional studies showed in the patient's fibroblasts a significant decrease in Nesprin-1, changes in nuclear morphology and LINC complex defects. Reverse phenotyping revealed 22 undiagnosed patients with two or more *SYNE1* missense variants, and 10 additional undiagnosed patients with at least one of the variants from the index case. All patients exhibited epilepsy-associated features or neurodevelopmental phenotypes.

Conclusion: This study highlights the current limitations for predicting how missense variants affect both protein function and their potential pathogenicity. These challenges are particularly pronounced in large proteins like Nesprin-1, where it is difficult to investigate the structural implications, especially given the multiple isoforms expressed from the *SYNE1* gene. Prioritizing the patient's phenotype/genotype correlation and conducting functional studies have been crucial for reaching an etiological diagnosis. Furthermore, understanding the effect of *SYNE1* missense variants would potentially identify novel associated phenotypes.

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P15.114.A Identifying druggable somatic variants in patients affected by lymphatic vascular malformations in a monocentric cohort

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Background/Objectives: Vascular malformations define a heterogeneous group of conditions characterized by a wide spectrum of clinical phenotypes ranging from mild forms such as isolated capillary malformations to severe forms involving multisystemic districts and vital organs such as lymphatic malformations[1]. While molecularly targeted therapies can be lifesaving, identifying the molecular cause is often challenging due to complex access to affected tissue samples, mosaicism, or insufficient sequencing depth[2,3,4].

Methods: Among 28 patients, DNA was extracted from affected tissue (fresh biopsy or formalin fixed paraffin embedded (FFPE)) in 16 individuals. The analysis was performed on DNA extracted from fresh biopsy (4/16), on FFPE 11/16 and from cystic fluid (CF) in one. Tissue somatic libraries were prepared with Myriapod® NGS Cancer Panel DNA (Diatech Pharmacogenetics s.r.l.) and sequenced in platform Mi-Seq® System (Illumina), ensuring average coverage per sample of 3000X.

Results: Pathogenic *PIK3CA* variants were identified in 11/16 patients, with VAF ranging from 1.16% to 7%. Variants in multiple targetable genes (*BRAF and PIK3CA*) were found in one individual. The test was negative in 5/16 patients: one screening failure, three not informative results and one with only CF available.

Conclusion: In total, we identified druggable variants in 68% of patients with low level of mosaicism using a multi-tier genetic approach. Given the recent advances in treatment, targeted therapy was applied with beneficial outcome in three challenging patients not responding to previous treatment. Our study confirms that identification of the molecular cause for complex vascular anomalies is fundamental and needs to be prioritized whenever possible.

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P15.115.B Targeted long-read sequencing for inherited cancer diagnostics

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Background: Precise detection of pathogenic variants in genes implicated in hereditary cancer is important for accurate diagnosis and patient management. This study evaluates the efficacy of targeted long-read sequencing (TLRS) with a custom designed panel, including genes relevant for inherited cancer diagnostics and variants that make up an established poly-genic risk score (PRS) for inherited breast-cancer. The targets include *PMS2*, a gene that is challenging to sequence with short reads due to pseudogene interference.

Material and Methods: A custom sequencing panel was developed, covering both the 313 variants of the PRS and the whole gene segments (+/− 5 kb) around 31 genes (Twist Bioscience). Ninety-six individuals with suspected hereditary cancer were included in a pilot study to assess the performance of the panel. Sequencing libraries were constructed using the Twist long read library preparation and standard hyb v2 enrichment and sequenced on the PacBio Revio system. An inhouse bioinformatics pipeline was developed and identified variants were interpreted using Alissa Interpret software (Agilent Technologies).

Results: Benchmarking analysis showed high F1-scores for both SNVs (99.96%–99.83%) and indels (93.47%–93.01%) in NA12878 and NA24385 GIAB samples. The TLRS panel had good coverage depth and uniformity across all targets. Reads were able to align accurately and reproduce variants across all genes including *PMS2*. The long-read approach also allows for variant phasing.

Conclusion: TLRS provide great sensitivity for targeted gene panels, it helps resolve complex regions and provides information on compound heterozygosity. Our findings support the integration of TLRS in the clinical routines.

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P15.117.D The clinical utility of ultra-long Nanopore Adaptive Sampling for rare disease investigation including NGS intractable regions

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Background: Many rare disease patients remain usolved after current comprehensive genetic testing, partly due to the complexity of certain genomic regions and limitations in current detection technologies. The opsin array on Xq28 represents one such region, and patients affected by X-linked cone-dysfunction have limited access to effective testing. Advances in nanopore long-read sequencing and computational approaches provide new opportunities to address these challenges. We investigated the utility of nanopore adaptive-sampling for variant detection across an inherited retinal dystrophy (IRD) gene panel including the opsin array.

Methods: Ten affected or carrier individuals (3 females) were recruited from Moorfield's Eye Hospital either with a known opsin array mutation or prior inconclusive testing. Fresh or frozen EDTA blood derived DNA was subjected to ultralong-read sequencing targeting a custom panel of 309 IRD genes by adaptive-sampling on the Oxford Nanopore Technologies PromethION platform. Sequencing runs were typically 12h and yielded 2.6–4.8 Gb of data with N50 ranging 33–61 kb.

Results: This approach enabled detailed characterization of single nucleotide variants (SNVs), the array composition, and hybrid gene combinations. Ten pathogenic mutations in 10 individuals were identified including SNVs, complete array deletion, and known pathogenic mis-splicing haplotypes in exon 3 including phased variant detection in three female carriers.

Conclusion: Ultra-long-read sequencing enables unambiguous read-through of large multi-copy gene arrays, with variant detection and phasing. Adaptive-sampling adds an effective targeting method avoiding complex enrichments. This study demonstrates the utility of our method for use in a diagnostic pipeline for patients and families with rare genetic disorders.

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A. Jestin: None. **S. Lin:** None. **T. Erlenbach:** None. **O. Mahroo:** None. **M. Michaelides:** None. **A. Webster:** None. **G. Arno:** None.

P15.118.A UDP-IST: First Undiagnosed Disease Program in Istanbul - Insights from Early Outcomes

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Background: In 2022, ACURARE launched ISTisNA Platform (https://istisna.org), a national initiative to improve the diagnosis and management of rare and undiagnosed diseases in Istanbul. Undiagnosed Diseases Program (UDP-IST), was implemented in 2024 as the first structured workflow for patients who remain undiagnosed despite previous evaluations. This study assesses the program's workflow, diagnostic strategies, and early outcomes.

Material and Methods: UDP-IST follows a structured approach where referring clinicians submit clinical and genomic data through an online portal. The ACURARE expert panel reanalyzes genomic data using an in-house bioinformatics pipeline and collaborates with referring clinicians for deep phenotyping. If cases remain unresolved, WGS and transcriptome analyses are performed. A dedicated team manages data analysis and case tracking.

Results: Between January-December 2024, 45 unsolved referrals were reviewed in biweekly multidisciplinary meetings. WES reanalysis solved 27% (12/45) of submissions without further testing. Repeated WES due to data quality solved 1 patient. WGS

provided diagnoses in five additional cases where WES was inconclusive. Two families underwent WGS for the index and WES for the parents, revealing a candidate variant in a novel gene in one family. Solved or unsolved, all families received a written report, and face-to-face genetic counseling was provided. Functional validation studies, including RNA sequencing and episignature analysis are ongoing for unsolved patients with VUS or negative results.

Conclusion: UDP-IST is an inaugurational model for Istanbul and Turkey. Successful integration of genomic reanalysis, expert case discussions, and functional studies are fundamental components. We hope our model paves the way for a nationwide implementation.

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P15.119.B Chromatinopathies: on the trail of the wheel

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Background: Chromatinopathies (CPs) are an expanding group of rare genetic disorders affecting epigenetic machinery. Beside an intricated genotypical spectrum, these conditions are also merged by overlapping phenotypes characterized by neurocognitive impairment, growth defects and distinctive, but often convergent, facial features. Due to their complexity and rarity, accurate diagnosis and management pose significant difficulties. Our goal is to gain a deeper understanding of this complex spectrum of conditions, aimed to ameliorate our diagnostic and managing approach to them and, prospectically, to identify novel therapeutic targets.

Material and Methods: We conducted a retrospective chart review of all patients diagnosed with CP seen at our Clinic before December 31st, 2024. Clinical data, including growth parameters, neurodevelopmental assessments, and facial photographs, were collected and analyzed.

Results: 235 individuals affected by 31 distinct CPs, mainly Sotos Syndrome, Rubinstein-Taybi Syndrome and Kleefstra Syndrome were enrolled. We compared diagnostic tests, analyzed growth parameters, deepen neurocognitive profiles and facial traits. Our data globally confirm the complexity of this spectrum of conditions, suggest the absence of pubertal peak in CPs and underline the burden of convergent phenotypes in this spectrum. Well-trained medical geneticists and tight collaboration with Laboratory play a key role to avoid diagnostic odyssey in CPs and to set up tailored health care.

Conclusion: This study represents the largest collection of CPs patients to date. Our data provide valuable insights into the clinical spectrum of these rare disorders, leading to improved diagnostic, therapeutic, and counseling strategies, paving the way for the development of personalized care plans.

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P15.120.C Deciphering the Genetic Enigma: The Paradox of Zero Phenotype-Genotype Correlation in ABCD1 Gene Mutations

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Background: X-linked adrenoleukodystrophy (X-ALD), caused by pathogenic variants in the **ABCD1 gene**, presents a perplexing clinical heterogeneity, where individuals harboring identical mutations exhibit **discordant phenotypic outcomes**, ranging from severe neurological impairment to an asymptomatic state. This **lack of genotype-phenotype correlation** complicates risk stratification and clinical management. Emerging evidence suggests that secondary molecular regulators may **modulate disease expressivity**. This study investigates the potential role of **SIRT1, PGC1α, GLUT21, RAGE, ABCD2, and GDF15** as candidate modifiers in individuals with **ABCD1 mutations**.

Material and Methods: A cohort of **eight genetically confirmed individuals** with **ABCD1 mutations** was recruited, comprising **four symptomatic and four asymptomatic individuals**. Peripheral blood mononuclear cells (PBMCs) were analyzed using **flow cytometric assays** to quantify the expression levels of **SIRT1, PGC1α, GLUT21, RAGE, ABCD2, and GDF15**. Statistical analyses were performed to assess differential expression between symptomatic and asymptomatic groups.

Results: A significant **downregulation** of **SIRT1, PGC1α, GLUT21, RAGE, ABCD2, and GDF15** was observed in symptomatic individuals compared to asymptomatic carriers ($p < 0.05$). The reduced expression of these markers implicates a dysregulation in **mitochondrial function, metabolic homeostasis, oxidative stress response, and cellular signaling**, which may contribute to the variable disease phenotypes.

Conclusion: These findings suggest that **SIRT1, PGC1α, GLUT21, RAGE, ABCD2, and GDF15** act as potential **phenotypic modifiers** in X-ALD, influencing disease severity independently of ABCD1 mutation status. Understanding these molecular pathways may provide insights into **biomarker-based risk assessment** and **targeted therapeutic strategies**, refining precision medicine approaches for X-ALD management.

C.O.I. Disclosure:

S. Kumar: A. Employment (full or part-time); Modest; Scientist.

P15.121.D Investigating the clinical and genetic landscape of *MORC2*-related disorders

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Background: Pathogenic missense variants in the *MORC2* gene are associated with two distinct disorders: Charcot-Marie-Tooth disease type 2Z (CMT2Z), a hereditary neuropathy, and DIGFAN syndrome, which has a broader and more variable clinical spectrum.

Material and Methods: Clinical and imaging data from 12 patients with DIGFAN and 3 patients with CMT2Z were collected. For functional analysis, a MORC2 expression vector with a C-terminal Flag tag was constructed and transfected into HEK293T cells. Twelve missense variants were introduced into the wild-type vector, and Western blot analysis was performed to assess protein stability.

Results: A total of 10 different missense variants in the *MORC2* gene were identified in our cohort, including five novel variants. The analysis of clinical data revealed a potential correlation between specific *MORC2* variants and predominant clinical features. For example, the S87L and R132C variants were frequently associated with diffuse hypotonia and muscle weakness, while the E27K variant was linked to ataxic gait and other disturbances without severe muscle weakness. Protein expression levels of MORC2 were lower in DIGFAN patients compared to CMT2Z cases; however, this difference was not statistically significant and did not account for the observed clinical variability.

Conclusion: This study enhances the phenotypic understanding of DIGFAN syndrome. Although Western blot analysis showed no significant differences in protein expression between the two phenotypes, our findings suggest that specific *MORC2* variants may influence clinical manifestations. Further research is needed to elucidate the mechanisms underlying the phenotypic variability in *MORC2*-associated disorders.

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P15.122.A The minigene assay as a tool for evaluating the pathogenicity of genetic variants in hearing loss patients

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Background: The next generation sequencing (NGS) is becoming a common tool in clinical setting. It allows for quicker and more accurate diagnosis, especially in genetically heterogenous diseases as hearing loss (HL). This technique generates a huge number of variants, which creates a need for their functional verification. One such tools is the minigene assays, which allows for the assessment of the possible effect on splicing.

Material and Methods: Ten variants detected in HL patients from the Genetic Clinic of Institute of Physiology and Pathology of Hearing were selected to assess their pathogenicity using the minigene assay: *EYA1* c.1475+1G>T, *EYA4* c.1282-12T>A, *GSDME* c.991delT, *GSDME* c.1127A>G, *MYO6* c.816+1G>A, *MYO6* c.1984-1G>A, *MYO6* c.3281-13A>G, *MYO7A* c.2829G>A, *MYO15A* c.9230-4A>T, *SLC26A4* c.1001+1G>A. All variants were detected using a dedicated panel of HL genes (237 genes). The fragments of genes of interest were amplified from patients' DNA and cloned into the expression vector pDEST-pCI-Neo-RHO using the Gateway system and transfected into HEK293T cells. The transcripts were analyzed using RT-PCR and Sanger sequencing.

Results: Most of the studied variants (9 out of 10) affected splicing. The most commonly observed effects were exon skipping and intron exonization, mostly resulting in the creation of premature stop codon.

Conclusion: Performing the minigene assay allowed for the verification of the pathogenicity of all studied variants. Splicing may be affected not only by variants disrupting the canonical splice sites but also coding variants and those located deeper within the intron sequence. Both syndromic and isolated HL can be caused by splicing affecting variants.

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P15.123.B Autosomal recessive *TTN*-related disease: evaluating the need for partner screening

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Background: Heterozygous truncating *TTN* variants (TTNtv) are a common cause of autosomal dominant dilated cardiomyopathy, while autosomal recessive (AR) titin related diseases are rare and associated with severe early-onset cardiac and skeletal muscle phenotypes. Variant interpretation is challenging due to a carrier frequency of 1% in the general population. This study explores the need for partner screening of TTNtv carriers to guide reproductive planning.

Material and Methods: A literature review was conducted using PubMed and Embase to identify studies describing patients with biallelic *TTN* variants. Additionally, we analyzed TTNtv in UK Biobank participants.

Results: The literature review encompassed 28 articles, identifying four AR phenotypes: congenital titinopathy, Salih myopathy, proximal titinopathy, and distal titinopathy. A total of 114 unique *TTN* variants were reported in 112 patients (38% frameshift, 38% nonsense, and 24% splice site). The I-band was most frequently affected (42%). Despite comprising only 6.7% of *TTN* coding sequence, the M-line accounted for 36% of reported variants. Analysis of 200,643 UK Biobank exomes identified 2,443 TTNtv, corresponding with a population carrier frequency of 1 in 82 (1.2%). The carrier frequency of TTNtv specifically within the I-band and M-line regions was approximately 1 in 106.

Conclusion: The high carrier frequency of TTNtv in the general population combined with uncertainty regarding their clinical

relevance do not support routine partner screening for all TTNtv. We propose to limit partner screening to carriers of known AR related *TTN* variants, in case of a familial history of TTNtv related disease or for TTNtv consanguineous couples.

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P15.124.C DoAggregate: a pipeline for aggregation testing for rare-variants on a gene and pathway level

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Background: Aggregation tests address the limitations inherent to the analysis of rare variants in cohort studies by allowing for the combination of multiple genetic markers within a genomic region. However, choosing the appropriate statistical test remains a challenge, as existing tools often fail to provide a comprehensive solution that encompasses automatic data processing, gene-level testing, and exploration of broader genetic regions such as pathways.

Material and Methods: To address these questions, we developed DoAggregate, an innovative, scalable pipeline designed for high-performance computing environments. DoAggregate processes lists of annotated genetic variants in TSV or GVCF formats, supporting tasks ranging from data filtering through pathway analysis to identification of regions of interest, including genes and pathways. It incorporates Excalibur, an ensemble method combining 36 aggregation tests, enhancing analysis by presenting detailed results in Excel format with annotations and plots for nuanced interpretation. The pipeline supports parallelization via Slurm, requires minimal input, and provides extensive resources for data preparation and analysis.

Results: DoAggregate is a comprehensive pipeline for aggregation testing that facilitates in-depth exploration of genetic regions across genomes and exomes. By combining detailed annotations, robust statistical frameworks and user-friendly outputs, DoAggregate improves the interpretation of genomic data.

Conclusion: Our results demonstrate its effectiveness in uncovering disease-related genetic architectures, accelerating biomarker discovery and paving the way for new therapeutic targets, making it an indispensable tool for genomic researchers.

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P15.125.D Genetic basis of internal tumors in Xeroderma pigmentosum

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Xeroderma pigmentosum (XP) is a rare, autosomal recessive genodermatosis caused by Excision Nucleotide Repair deficiency. These patients are sensitive to sunlight, leading to an increased frequency of skin cancers. Recently, it was found that some XP patients are at a very high risk of developing internal tumors, including central nervous systems, hematological malignancies, gynecological and thyroid cancers. We analyzed four published, clinically well-defined XP cohorts (from the States, France, England and Brazil) and assessed the cancer risk of these patients. The Odds Ratio (OR) are very high for young XPs (more than a thousand times higher than for the general population) for the 0–20-year-old French XPs. Very high OR are also observed for English, French, and American XPs for developing brain tumors, hematological malignancies, and thyroid tumors. We analyzed, by whole genome sequencing, 18 internal tumors from XP patients, mainly hematological malignancies. Results demonstrated a dramatic increase in mutation rates in XP-C tumors versus sporadic tumors. A strong mutational asymmetry with respect to transcription and level of gene expression demonstrated the existence of transcription-coupled repair in vivo in humans. Mutations appeared to be caused by unrepaired bulky guanine lesions on the untranscribed strands of DNA. The mutational profiles of internal XP-C tumors are specific to NER- deficiency and nearly identical between all XP-C internal tumors regardless of the tumor type. Physicians following XP patients should be aware of the high risk of internal tumors. Prevention and early detection of leukemia and brain, gynecological and thyroid tumors are needed.

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P15.126.A Frequency of Bardet-Biedl syndrome variants in a population with early-onset obesity

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Background: The melanocortin-4 receptor (MC4R) pathway is crucial for hunger regulation, energy balance, and weight control. In patients with Bardet-Biedl syndrome (BBS), dysfunctional primary cilia impair the MC4R pathway, causing symptoms like vision loss, learning difficulties, renal dysfunction, hyperphagia, and early-onset obesity. The Rare Obesity Advanced Diagnosis (ROAD)™ program aims to improve genetic testing access for

suspected rare MC4R pathway diseases in Europe and the Middle East.

Methods: Genes (*BBS1-BBS22*) from individuals with early-onset obesity were sequenced. Variants were classified as pathogenic/likely pathogenic/variants of uncertain significance (P/LP/VUS).

Results: Among 5,051 individuals, 16 individuals had a P/LP biallelic variant and 72 had a VUS biallelic variant, with *BBS14*, *BBS20*, and *BBS9* variants most frequent. For individuals <18 years, mean BMI z-score was 3.8; for ≥18 years, BMI was 41.6 kg/m^2. Age of obesity onset was 4.8 years and hyperphagia onset was 3.1 years. Individuals with P/LP/VUS variants were from Türkiye (5.82% of all individuals tested in the country), Spain (1.09%), Italy (1.08%), UK (2.25%), Israel (0.60%), Ireland (0.80%), and Germany (1.32%). Only 25 (28%) had a BBS suspicion at testing.

Conclusion: Among tested individuals, 1.74% with early-onset obesity had a biallelic variant in one of 22 BBS genes, with higher frequencies of *BBS14* and *BBS20* variants versus literature and a high frequency in Türkiye due to consanguinity. Not all patients had a clear BBS phenotype at diagnosis, indicating the need to include all BBS genes in early-onset obesity panels to improve disease understanding and early diagnosis for potential novel treatments.

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P15.127.B Investigating the molecular function of *THAP12* in neurodevelopment

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Background: Lennox-Gastaut Syndrome (LGS) is a severe epileptic encephalopathy presenting significant diagnostic and therapeutic challenges, with approximately 30% of cases remaining undiagnosed. These cases offer an opportunity to explore novel biological mechanisms underlying LGS. Whole genome sequencing in two patients with severe LGS revealed biallelic variants in *THAP12*, a gene encoding a zinc finger protein involved in DNA binding and transcriptional regulation. However, *THAP12* has not been previously associated with any neurological disorder, and its molecular function, particularly in neurodevelopment, is poorly understood. We hypothesize that THAP12 functions as a transcription factor regulating genes critical for neurodevelopment.

Materials and Methods: In our first objective, we will generate *THAP12*-KO in HEK293FT cells to understand the general role and function of THAP12 in-vitro. We will perform chromatin immunoprecipitation (ChIP-seq) followed by whole transcriptomic analysis (RNA-seq), to understand how target genes are differentially regulated by THAP12. We will identify potential interacting partners of THAP12 using co-immunoprecipitation (Co-IP) coupled with liquid mass spectrometry. In our second objective, we will repeat these experiments in neural progenitor cells to identify target genes and interacting partners of THAP12 contributing to its role in neurodevelopment.

Results: Our preliminary results from RNA-seq on *THAP12*-KO HEK293FT cells have revealed a significant dysregulation of genes involved in neurodevelopment and transcription regulation. We have also identified several candidate interacting partners of THAP12 involved in cell cycle progression, neurodevelopment, and neurometabolism.

Conclusion: Our findings will shed light on *THAP12*'s role in neurodevelopment, offering insights into potential therapeutic targets for LGS.

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P15.128.C Limits and pitfalls of genome alignment tools: a case report

Francesca Cappuccini[1], *Laura Pezzoli*[1], *Daniela Marchetti*[1], *Camilla Lucca*[1], *Lucrezia Goisis*[1], *Chiara Mongodi*[1], *Nunzia Della Malva*[1], *Loredana Perego*[1], *Riccardo Rubini*[1], *Anna Rita Lincesso*[1], *Matteo Bellini*[1], *Agnese Scatigno*[2], *Lidia Pezzani*[2], *Erica Rosina*[1], *Davide Tonduti*[3,4], *Fiammetta Zunica*[5], *Maria Iascone*[1]

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Background: We report on a 16-year-old female third child from consanguineous parents (first degree cousins). The patient's mother was affected by systemic lupus erythematosus and hypothyroidism and the patient brother had chilblain lupus. The sister and the father were reported healthy. The patient presented at 15 years old with chilblain lupus, mitral valve insufficiency. Interferon signature was tested positive.

Material and Methods: We analyzed the patient-parents trio by PCR-free 2 × 150 bp Whole Genome sequencing; bioinformatics analysis was performed with Dragen version 3.9.5. As a validation, we performed RNAseq from PAXgene samples of peripheral blood and Sanger sequencing after RT-PCR.

Results: From trio-WGS data we suspected the presence of a homozygous duplication of the region chr20(GRCh38):g.36930231-36932015, encompassing exon 5 of the *SAMHD1* gene. Indeed, the bioinformatic pipeline called a 1784 bp heterozygous duplication within a run of homozygosity in proband and both parents, but visual inspection of the BAM files suggested the duplication would be homozygous in the proband and heterozygous in both parents. By RNAseq we confirmed the tandem duplication of *SAMHD1* exon 5, but we needed to perform RT-PCR and Sanger sequencing to confirm the homozygosity in the proband and heterozygosity in

parents. The duplication is predicted to generate a frameshift and is classified as likely pathogenic.

Conclusion: We highlight a main limitation of alignment tools in identifying homozygous duplications and we would suggest paying particular attention to isolated heterozygous calls within runs of homozygosity, since they could represent the only alert to address the right finding.

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P15.129.D Artificial Intelligence and Facial Analysis for Diagnosing and Genotype Phenotype Correlation in RASopathies

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Background: RASopathies are a group of developmental syndromes caused by germline mutations in genes belonging to the Ras/MAPK pathway. Common features include intellectual disability, congenital heart defects, skin abnormalities, and craniofacial abnormalities. Due to their high genetic variability, establishing a genotype-phenotype correlation can be challenging. We aim to assist in diagnosis and genotype-phenotype correlation by using artificial intelligence and facial images.

Material and Methods: We tested AIDY (Artificial Intelligence for Dysmorphology), an artificial intelligence tool developed at Institut Imagine, Necker-Enfants Malades Hospital. We retrospectively included all frontal and lateral pictures of patients with a molecular diagnosis of RASopathies. We tested AIDY's ability to provide an accurate diagnosis.

Results: In total, we identified 197 patients in our local database with molecularly confirmed RASopathy and facial pictures. Of these, 73% (N = 143) had Noonan syndrome, 11% (N = 22) had neurofibromatosis-Noonan syndrome; 7.6% (N = 15) had Costello syndrome; 3% (N = 6) had an indistinguishable phenotype between Noonan syndrome and cardiofaciocutaneous syndrome, 2.5% (N = 5) had cardiofaciocutaneous syndrome, 2% (N = 4) had Noonan syndrome with multiple lentigines. Computational analysis is still ongoing, and we are hoping to have results soon.

Conclusion: Next-Generation Phenotyping using artificial intelligence could be a real benefit for patients, helping establish a precise diagnosis and genotype-phenotype correlation. Indeed, some genes are more associated with certain features; for example, RIT1 and RAF1 are associated with cardiomyopathy, and PTPN11 and KRAS with the risk of cancer.

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P15.130.A Application of Nanopore sequencing for CNVs characterization in paediatric patients with genetic epilepsies

Simona Virdò[1], *Elena Cellini*[1], *Flavia Fineschi*[1], *Valentina Cetica*[1], *Aurelia Iuliana Timis*[1], *Dalila De Vita*[1], *Domenico Rutigliano*[1], *Daniela Pucatti*[1], *Claudia Mandorlini*[1], *Renzo Guerrini*[1,2], *Elena Parrini*[1], *Davide Mei*[1]

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Background: Genetic epilepsies (GEs) represent a group of conditions caused by heterogeneous genetic aetiologies. Long-read sequencing enables precise detection and characterization of copy number variants (CNVs) and complex rearrangements, increasing diagnostic yield in unsolved cases. We utilized Oxford Nanopore Technologies (ONT) long-read sequencing on a cohort of paediatric GE patients with pathogenic CNVs, previously identified using conventional methods, to enhance their molecular characterization.

Material and Methods: We re-analysed the DNA of patients carrying CNVs previously detected by Multiplex ligation-dependent probe amplification (MLPA) or NGS sequencing. We performed whole genome or adaptive sampling sequencing, a software-based enrichment targeting the regions of interest during sequencing, using PromethION or MinION flow cells.

Results: Nanopore sequencing allowed to confirm and better characterize two intragenic deletions involving the DEPDC5 and STXBP1 genes. An alleged duplication, including four SCN1A exons detected by MLPA, was reclassified as a complex intragenic rearrangement resulting from a flanking duplication with multiple deletions. We also defined the exact breakpoints of two deletions involving the SCN1A gene; in one patient, we unravelled the additional involvement in the deletion of a gene located upstream SCN1A.

Conclusion: ONT long-read sequencing has proven to be diagnostically and research-wise useful, enabling the validation of known CNVs, allowing a precise identification of breakpoints, and improving the refinement and characterization of complex rearrangements in GEs. Our findings demonstrate the effectiveness of this platform in the diagnostic workflow for characterizing genomic abnormalities and elucidating their role in the disease pathogenesis, providing significant clinical insights.

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P15.131.B Understanding the role of a protein subunit in an undiagnosable neurodevelopmental disorder

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Background: We report a consanguineous Pakistani family where a male and female cousin had an undiagnosable neurodevelopmental disorder. Both had a similar phenotype; severe

developmental delay, cerebellar anomalies, insensitivity to pain, and a progressive sensory and motor peripheral neuropathy that affected arms more than the legs. We aimed to identify the genetic cause and molecular mechanisms underlying this disease.

Material and Methods: We undertook whole exome sequence analysis and identified a potential mutation in the *RPP30* gene. RPP30 is a subunit of the ubiquitous ribonuclease P complex. We carried out cellular and molecular studies to understand the contribution of this mutation in this disorder.

Results: The mutant RPP30 forms aggregates in the nucleus unlike the wild-type protein which is diffused. The mutant protein has an overall reduced cellular expression, and is degraded by the proteasomal pathway. The mutant RPP30 still interacts with a second partner protein, which, along with several other proteins, forms a functional ribonuclease P complex. Curiously, in the presence of this second protein, the mutant RPP30 forms less cellular aggregates.

Conclusion: We identified a new gene, *RPP30*, as a novel cause of a rare neurodevelopmental disorder. Functional studies indicate that the mutation identified in the patient likely disrupts the protein structure and function, leading to the disease.

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S. Roy Choudhury: None. **C. Woods:** None.

P15.132.C Implementation of rare diseases registry into hospital

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Background: In the European Union, a rare disease (RD) is defined as one affecting fewer than 5 out of 10,000 members of the general population. Only approximately 350 RD have an ICD-10 diagnosis code, which is why patients cannot be identified in patient information systems and thus RD prevalence and patients' numbers cannot be estimated.Tartu University Hospital (TUH) is the only university hospital in Estonia and in certain fields the only center of expertise. The Rare Disease Competence Centre (RDCC) is established at the TUH IN 2021. The RD registry (RDR) is part of the TUH's e-health system, data about RD patients is registered here.

Material and Methods: TUH is the first hospital to implement RDR in Estonia from April 1st 2023. Data about RDCC patients' were registered retrospectively from the year 2012 and prospectively. The data is collected according to the Set of Common Data Elements for RD Registration. The information collected refers to ORPHA-code, ICD10 code, disease history and information about the laboratory analysis on the basis of which the diagnosis has been confirmed.

Results: To January 26th 2025, the registry includes 2746 entries of patients with all cytogenetically and molecular-genetically confirmed RD. 832 different ORPHA-codes have been used. Among them, 37% of patients (997) are pediatric <10 years, and 14.% (390) of them are hereditary cancer syndrome patients.

Conclusion: The systematic collection of data on patients with RD provides important information on both the prevalence of diseases and the number of patients.

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P15.134.A Novel Illumina 5-base NGS approach can bring genomic and epigenomic findings using single assay

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Multiomics studies provide an integrated approach to identify complex biomolecular fingerprints that may indicate disease. For example, the analysis of genomic variants in combination with epigenetic signatures allows the interrogation of a wide spectrum of disease-associated loci for a variety of conditions such as Rare Undiagnosed Genetic Diseases (RUGD) diagnostics and cancer classifications. This approach typically employs individual, disparate assays that provide single-omic information, creating a barrier that is often costly and time-consuming. A single platform that provides a readout of both genomic and epigenomic analysis could substantially simplify our ability to collect and analyze complex multiomic information.Here we present novel, single-step methyl-conversion library-prep technology capable of simultaneous genome variant detection and DNA methylation profiling in a single experiment. It is a simplified workflow compared to commercially available conversion technologies, resulting in a less than seven-hour turnaround time, while also requiring as little as 50 ng of genomic DNA input.We evaluated our solution against current methods for DNA sequencing and methylation analysis including: Whole Genome Sequencing, Enzymatic Sequencing (EM-Seq) and Illumina Methylation arrays. We show that the single 5-base solution presented can produce results that can be used for disease cases resolution.

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P15.135.B Genome sequencing (GS) vs targeted gene panel next generation sequencing (panelNGS) for paediatric and syndromic cardiomyopathy in the diagnostic setting:

resolution, reanalysis, and review

Phillipa May[1], *Matthew Edwards*[1], *Lizi Briggs*[1], *Maha Younes*[1], *Andrew Fleming*[1], *Renata Gomes*[1], *Anna Lockhart*[1], *Sally Walsh*[1], *Krunal Mahida*[1], *Sam Wilkinson*[1], *Amy Slater*[1], *Katie Williams*[1], *Steven Muller*[1], *Tahni-Ann Wilson*[2], *Ellie Quinn*[2], *Tessa Homfray*[1], *Deborah Morris-Rosendahl*[1]

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Background: We present a two-year retrospective analysis of genetic diagnostic testing for paediatric or syndromic cardiomyopathy, to determine the 'added value' of GS.

Material and Methods: Patients were referred for a custom NGS panel (n = 100+ probands) or singleton/duo/trio GS performed in the NHSE Genomic Medicine Service (80+ probands). Both approaches use a bioinformatically-applied gene list (119–160 genes in PanelApp), however panelNGS was mostly restricted to protein-coding regions.

Results: GS identified a genetic aetiology fully explaining the patient's cardiac phenotype (likely pathogenic or pathogenic variants) consistent with the inheritance pattern, in 41% of cases versus 18% of panel NGS cases. The panelNGS yield was increased to 20% following further testing such as parental testing. The GS approach resulted in fewer follow up tests and quicker resolution. We dissect the reasons for this difference, breaking down resolution rates in families versus singletons, and the location of variants not detectable by panelNGS. Panel App is regularly reviewed and our data show the success of incremental changes. We compare the Tiering approach for filtering variants to Exomiser and examine the effects of the number of Human Phenotype Ontology (HPO) terms submitted with the referral.

Conclusion: In a phenotypically heterogenous patient cohort, GS has advantages over targeted panel testing, allowing analysis of variation outside the standard target: genes not included in the panel, non-coding regions and the identification of structural variants. However, these advantages depend on efficient prioritisation of variants, which requires a well-structured bioinformatic pipeline.

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P15.136.C Deploying multiomics and pangenome tools to detect complex variants, validate VUS, and increase testing equity in rare disease

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Background: We evaluated the ability of nanopore-based long-read sequencing (LRS) to detect clinically relevant complex variants in a cohort.

Material and Methods: We targeted outputs practical and cost-effective for a clinical laboratory setting (e.g. no ultra-long reads).

Results: In Y-X translocations, alignment to the T2T reference disambiguated the pseudoautosomal regions and revealed different breakpoints as well as additional X deletions undetected by clinical testing. In 46,XY gonadal dysgenesis caused by 9pter deletions, we show that the terminal deletions are in fact exchanges of telomeric regions with another chromosome. To genotype segmentally duplicated regions, such as the RCCX module associated with Congenital Adrenal Hyperplasia, *TNXB*-associated Ehlers-Danlos syndrome, and susceptibility to schizophrenia and autoimmune disorders, we created a new tool, *Parakit*. Creating an enriched local pangenome, Parakit mapped and phased variants and fusions, counted modules, and made diagnoses in singleton-only cases, beyond the ability of current clinical assays. Diagnosing singleton-only cases is critical to addressing inequity in genetic testing for the millions of families where one or both biological parents are not available. By systematically deploying RNA sequencing in parallel with genome sequencing, we demonstrated non-canonical exon inclusions as a frequent etiology in rare disease. But =, in addition, the CpG methylation status available in the LRS dataset showed an episignature matching that of CSS1 in a proband with a deep intronic VUS in *ARID1B*, leading to a reclassification of the variant to likely pathogenic and a firm diagnosis.

Conclusion: Deploying LRS and multi-omics reveals complexity and hidden diagnoses in rare disease.

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P15.137.D Exploring the neurophysiologic and neuropathologic basis of visual deficits in cystinosis

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Cystinosis is a rare, childhood lysosomal storage disorder caused by mutations in cystinosin, the transporter responsible for cystine from the lysosome to the cytosol. In cystinosis, mutated cystinosin causes cystine crystal accumulation and cell death. Photophobia, or abnormal sensitivity to light, is an early symptom of the disease and can be debilitating for patients. Historically, this photophobia has been attributed to ocular corneal cystine crystal accumulation, but eyedrops prescribed to disrupt these crystals do not fully alleviate symptoms. Furthermore, photophobia is known to have a neurobiological basis that extends beyond eye injury alone. Interestingly, when individuals with cystinosis were presented with a series of visual stimuli, they had increased early, cortical visual evoked responses. Given these data, we hypothesize that photophobia in cystinosis could be due to a central nervous system (CNS) hyperexcitability that results from prolonged corneal cystine accumulation. To explore this, we have developed a cystinosin knockout (*Ctns* $^{-/-}$) mouse model using CRISPR/Cas9. This model has been confirmed to produce a truncated, nonfunctional protein product. Phenotypically, our model recapitulates the human disease and demonstrates corneal eye crystal accumulation. Our model will be used to conduct similar visual electrophysiology studies to those conducted in patients and probe for a mechanism of cortical hyperexcitability using various neuromarkers. By recording similar neurophysiologic signatures across species and correlating them with changes in molecular signatures, we aim to deepen our understanding of the pathophysiology of photophobia in cystinosis and develop new therapeutics for individuals.

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P15.138.A Applications of ONT long read sequencing in rare disease research and diagnosis

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Background: Rare diseases, a diverse group of genetic disorders, affect a small portion of the population and are challenging to study due to their complexity. Traditional short-read sequencing (SRS) has limitations in diagnosing these conditions, especially in resolving genetic variants in repetitive regions. Recently, long-read sequencing (LRS) technologies, like those supported by Oxford Nanopore Technology (ONT), have become vital for addressing these challenges.

Material and Methods: This study employed ONT sequencing to analyze genomic DNA from patients with suspected hereditary cancer predisposition. The WholEx Pro protocol (IVD-CE, 4Bases Switzerland) was used for library preparation and sequencing, ensuring high-quality results. LRS generates reads up to 10 kilobases, enabling the detection of diverse genetic alterations, including point mutations, repeat expansions, large insertions, and structural variations. Results were validated through comparative analyses with data from other diagnostic laboratories, focusing on mutations in repetitive and homologous genomic regions that are difficult for SRS to resolve.

Results: Since October 2024, ONT has been actively utilized in our diagnostic lab. Initial analyses demonstrated the platform's accuracy in identifying pathogenic variants, including complex structural variations and repeat expansions. Cross-validation with external facilities confirmed the reliability of the workflow.

Conclusion: Integrating ONT sequencing has significantly improved variant detection in complex genomic regions, particularly in repetitive sequences. This advancement establishes ONT-LRS as a robust, efficient approach for diagnosing hereditary cancer syndromes, offering clear advantages over traditional short-read methods.

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P15.139.B Advancing precision medicine: long-read sequencing in complex variant identification

***Pasquale Di Letto**[1], Mariateresa Zanobio[1], Margherita Scarpato[1], Federica Romano[1], Sarah Iffat Rahman[1], Pasquale Primo[1], Maria Elena Onore[1], Rossella Nicoletta Borrelli[1], Carmine Spampanato[1], Giulio Piluso[1], Annalaura Torella[1,2], Vincenzo Nigro[1,2]*

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Background: Long-read sequencing technologies have revolutionized genomics by enabling the comprehensive detection of complex genetic variations, such as structural variants, repetitive regions, and intricate genomic rearrangements. Thus, they significantly enhance diagnostic capabilities for genetic disorders.

Material and Methods: Analysis was performed using Oxford Nanopore Technologies (ONT) and validated with Sanger sequencing or array-CGH.

Results: Firstly, we performed long-read sequencing to characterize complex variants for which short-read exome sequencing and bioinformatic analysis had initially suggested the presence of structural rearrangements not further defined. ONT confirmed these rearrangements and provided high-resolution breakpoint characterization. Given this, we present three cases in which long-read sequencing brought definitive diagnoses that conventional techniques overlooked. The first case involves a 447 bp Alu-mediated insertion in the NSD1 gene, resulting in exon 13 skipping, which induces the development of Sotos syndrome. In the second case, a 3 kb Alu-mediated deletion in the DEGS1 gene, associated with hypomyelinating leukodystrophy, was successfully identified after eluding detection by short-read sequencing and array-CGH analysis. The third case highlighted a complex maternal mosaicism in the DES gene resulting in two different deletions and disease manifestations, in an affected mother and child.

Conclusion: The cases presented highlight the transformative impact of long-read sequencing in clinical diagnostics. By uncovering previously undetectable genetic variations, this technology significantly enhances the accuracy of diagnoses, leading to deeper understanding of genetic disorders and more precise, personalized patient care. Its integration into clinical practice is not just beneficial but essential for advancing genomic medicine, improving disease management, and ultimately enhancing patient outcomes.

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P15.140.C Towards multiomics in rare disease: long-read and RNA sequencing complement short-read genome sequencing

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Background: Short-read genome sequencing (WGS), combined with bioinformatic tools for detecting repeat expansions, structural variants, and copy-number variants, enables high diagnostic yields across rare disease patient groups. However, not all genetic variants can be fully resolved or clinically interpreted using WGS alone. Such cases include structural rearrangements, repeat sequences, and variants located in non-coding regions. We have gathered several examples of these variants and explored the clinical value of complementary multiomic analysis.

Methods: WGS, long-read sequencing, RNA-sequencing.

Results: 1) A patient with clinical phenotype of Mb Osler harbored a variant in *ACVRL1* (MIM 600376). Long-read sequencing detected a 2.2 kb exonic insertion of mitochondrial DNA. The variant was deemed pathogenic as it disrupts an autosomal dominant disease gene matching the clinical phenotype. 2) In a newborn boy blind from birth clinical WGS did not identify any causative variant. RNA-sequencing identified loss of *PRICKLE3* expression. Reanalysis of WGS data on the child and the

unaffected mother revealed a hemizygous deletion in a transcription factor binding motif at the *PRICKLE3*-promoter. The variant is likely to cause the loss of expression but further functional studied are needed to prove causality of *PRICKLE3*. 3) In an individual with frontotemporal dementia but normal ELISA results, clinical WGS detected a likely pathogenic canonical splice-site variant in *GRN* (MIM 607485). Follow-up RNA sequencing revealed aberrant splicing caused by a cryptic splice site and not the typical haplo-insufficiency.

Conclusion: Long-read and RNA-sequencing can provide additional result to clinical analysis and thereby increase diagnostic yield

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P15.141.D Individual competence assessment of WGS/WES proportionate variant analysis using the GENie platform

Miranda Durkie[1], *Dave Cregeen*[2], *Angie Kolozi*[2], *Joseph Leslie*[3], *Ian Berry*[4], *Karen Stals*[5], *Martina Owens*[5], *Rachael Mein*[6], *Andrew Parrish*[5], *Sian Ellard*[5,7], *Emma Baple*[8,7,5], *Sandi Deans*[2]

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Background: Genomic diagnostic testing is increasingly expanding to whole exome (WES) and whole genome sequencing (WGS) to improve sensitivity for causative variants. However, the overall burden of variants requiring analysis is increased compared to targeted panels. To maximise variant scientist resource and capacity, proportionate analysis of variants, excluding those with low probability of pathogenicity and focussing on the most likely causative variants is recommended (PMID: 39327040 and https://www.youtube.com/watch?v=vOV_xiccw3U).

Material and Methods: GENie is an online, individual learning and assessment tool from GenQA. To support the genomics workforce, the pilot program included six clinical WES/WGS case scenarios that require variant prioritisation prior to further analysis and reporting. Participants assessed the annotated variants and chose to "exclude from analysis", "definitely report" or flag for "further variant classification". Upon result submission, expected responses by expert advisors were provided. Summary results will be presented at an online webinar prior to the introduction of the full GENie module.

Results: Results of this pilot scheme will be presented, focussing on learning points from difficult cases and variants, as well as participant summary data such as role, experience and time taken to complete each case.

Conclusion: The GENie online training platform allows scientists to showcase their expertise and continuing professional development. It can be used to evidence the competency of individual scientists to accurately and efficiently perform WES and WGS analysis, maximising resources to ensure patients get their results in a timely manner.

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P15.142.A Phenotype-driven filtering in whole exome sequencing: Results of Human Phenotype Ontology usage to create patient-specific virtual gene panels in a single center

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Background: Whole exome sequencing (WES) is widely used in rare disease diagnostics, yet its diagnostic yield remains suboptimal. Genomic variation must be filtered and analyzed, incorporating phenotypic data. Accurate phenotypic descriptions are essential in this process and the Human Phenotype Ontology (HPO) provides a standardized, hierarchical vocabulary, along with gene-phenotype associations.

Material and Methods: The results of 176 unrelated cases who underwent WES between 2019 and 2024 were retrospectively analyzed. Analyses involving known relatives were excluded. Phenotypic information was obtained from requesting physicians, and corresponding HPO terms were determined by clinical geneticists. Only variants associated with HPO terms were filtered, similar to a virtual panel specific to the patient, and diagnostic yield was examined.

Results: 981 (439 unique) HPO terms were assigned to 176 cases presenting with a broad spectrum of symptoms. The average per case was 5.57, with a median of 5 terms. Among them, 102 cases had ≥5 terms, while 74 cases had four or fewer. A definitive diagnosis was established in 56 cases (31.8%). Every diagnostic variant matched with at least 1 HPO term. There was no statistically significant difference in diagnostic rate between cases with ≥5 terms (31.4%) and with ≤4 terms (32.4%) ($p > 0.05$).

Conclusion: This study demonstrates that HPO-based filtering is highly effective. Unexpectedly, adding more symptoms does not necessarily increase the likelihood of diagnosis, which may be due to cohort size. Remarkably, even a single, well-chosen HPO term can be sufficient for diagnosis, as specificity plays a crucial role in identifying the causative variant.

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P15.143.B Targeted long-read sequencing of the RCA cluster to personalise management of aHUS

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Background: Atypical haemolytic-uremic syndrome (aHUS) is a rare life-threatening disease caused by genomic variation in the complement region. For approximately 20% of familial and 40% of sporadic cases, a molecular diagnosis is not established using standard sequencing methodologies due to complex surrounding genomic architecture. Accurate diagnosis is important, with drug treatments of £350k per patient/annum, and genetic causes predicting outcomes in transplants and disease relapse after treatment withdrawal.

Material&Methods: Our study aims to combine accurate target-capture and long-read sequencing for improved diagnostics. A hybridization capture panel was designed and optimised to target a 1 Mb genetic region required for aHUS diagnosis. A pipeline was constructed for a bespoke, cost-effective, long-read sequencing approach that would advance analysis of this structurally complex region.

Results: Using multiplexed sequencing on a single PromethION flowcell we demonstrate >90% capture and achieve up to 400x coverage per sample, with high-quality MAPQ 60 mapping versus low-quality MAPQ 0–20 in short-read data of the same region. Early analysis of 120 mutation positive cases confirmed reliable detection of all causal point mutations using the new approach, with improved structural variant interpretation, including precise breakpoints and greater resolution for complex structural rearrangements. Over 500 mutation positive cases and unresolved cases are now being assessed.

Conclusion: This pilot study validates a cost-efficient advancement for aHUS diagnostics. We aim to establish this novel genetic assay for the NHS. Accurate and rapid genetic diagnosis is essential for clinical decision-making and the approach can be easily expanded for other rare diseases caused by variation in complex regions.

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P15.144.C Definition of rare mutations involved in the pathogenesis of sporadic brain malformations using whole exome sequencing

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Background: Brain arteriovenous malformations (AVMs) are a significant cause of hemorrhagic stroke in both children and adults. AVMs are classified into hereditary and sporadic forms, with approximately 95% being sporadic and only about 3% hereditary. Despite extensive research, the mechanisms underlying brain AVM formation remain poorly understood.

Material and Methods: The study included a 39-year-old male patient diagnosed with brain AVM, confirmed via magnetic resonance angiography. DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen). Paired-end libraries were obtained by the Twist Library Preparation EF Kit and sequenced on the NovaSeq 6000 Illumina platform. Data analysis and annotation were performed using the GATK workflow, and variant call format (VCF) files were generated. To visualize and functionally group genes with filtered variants, the ClueGO plug-in in Cytoscape software was utilized. Candidate gene prioritization was carried out using ToppGene (https://toppgene.cchmc.org/). Variants were validated by Sanger sequencing.

Results: A total of 45,593,698 reads were obtained, of which 92.82% had a Phred quality score >30. After applying filtering criteria (Missense, nonsense, frameshift, and short indels; MAF <0.01), approximately 316 variants were selected. ClueGO enrichment analysis identified pathways related to cell-cell adhesion, telencephalon cell migration, and forebrain cell migration. Prioritization analysis highlighted key genes involved in biological processes, including aorta morphogenesis (*COL3A1*), BMP signaling pathway (*MAPK*), cellular response to endogenous stimuli (*CTBP2*), and positive regulation of intracellular signal transduction (*RNF31*).

Conclusion: We propose that disruptions in these pathways represent potential mechanisms contributing to the pathogenesis of brain AVMs.

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P15.145.D Novel EMC10 Frameshift Variant Detected by Whole Exome Sequencing: EMC10 Related Neurodevelopmental Disorder

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Background: The EMC10 gene, located on chromosome 19, encodes one of the 10 components of the endoplasmic reticulum complex (EMC), a highly conserved protein complex involved in membrane protein biology. EMC10-related Neurodevelopmental disorder (NDD) with dysmorphic facies and variable seizures is an autosomal recessive disorder characterized by global developmental delay apparent in early childhood.

Material and Methods: In the first step, peripheral blood samples were analyzed using karyotyping, FMR1 CGG repeats analysis, and microarray analysis. In the subsequent step, the patient underwent whole exome sequencing (WES). To confirm the detected variant and assess family segregation, Sanger sequencing was performed.

Results: Whole exome sequencing (WES) revealed a novel homozygous frameshift pathogenic variant (NM_206538.4) c.431del p.Asp144AlafsTer3 in the EMC10 gene, which was associated with the clinical presentation.

Discussion: A total of 31 cases, including ours, have been reported in the literature with this clinical presentation. In our case, developmental delay, speech delay, intellectual disability, poor weight gain, EEG abnormalities, dysmorphic facial features (long face, triangular face, long forehead, bifrontal narrowing, narrow nasal bridge, crowded teeth, full nasal tip, fifth finger clinodactyly), and microcephaly are present, which align with the characteristics observed in the other reported cases. However, our case does not exhibit kidney cysts, nephrocalcinosis, elevated

parathyroid hormone levels, ataxia, or hypotonia, which have been reported in other patients in the literature

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P15.147.B Genotype-phenotype spectrum of FBN1-related fibrillinopathies

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Background: Marfan syndrome (MFS) is an autosomal-dominant disorder with highly variable clinical manifestations including cardiovascular, ocular, hearing and a series of skeletal anomalies. Mutations in the gene for fibrillin-1 (*FBN1*) cause MFS, and over than 3700 pathogenic/likely pathogenic variants have been published to date. The aim of the present study was to identify the genetic diagnosis in a group of Bulgarian patients with symptoms strongly suggesting for MFS and to determine the contribution of rare *FBN1*-variants in the pathogenesis of the studied phenotypes.

Material and Methods: Thirty patients presenting symptoms, ranging from mild phenotypes, such as isolated ectopia lentis, retinopathy, or hearing impairment, to severe cardiovascular disorders, were recruited from different clinical departments. We performed WES on NovaSeq6000 platform of Illumina. Variants in MFS-associated genes were extracted from the WES data and filtered using systematic bioinformatics analysis and the American College of Medical Genetics and Genomics criteria.

Results: Fourteen disease-causing variants in *FBN1* were found in 16 out of 30 patients analyzed (53%) - 12 known and 2 novel. In 14 patients (47%) genetic cause of the disease was not identified, possibly due to the presence of non-coding mutations or copy number variation in related genes. Predominant clinical phenotype in the studied cohort was MFS/Marfan-like, but also non-syndromic retinopathy, primary open-angle glaucoma, ectopia lentis, and deafness.

Conclusion: *FBN1*-mutations have been found not only in MFS but also in a range of phenotypes, suggesting a role of this gene in the pathogenesis of wide spectrum of seemingly not-related diseases.

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P15.148.C High frequency of lysosomal storage disorders carriers in an Italian adult neurological cohort

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Background: Common neurodegenerative disorders (NDDs), such as Parkinson's disease (PD), Alzheimer's disease or ataxia of different aetiology, can present with symptoms similar to Lysosomal Storage Disorders (LSDs). A known association exists between *GBA* gene mutations at a heterozygous level and PD. Variants in the galactocerebrosidase (*GALC*), and arylsulfatase A (*ARSA*) genes, causing the autosomal recessive Krabbe disease and metachromatic leukodystrophy respectively, have also been identified as risk factors for NDDs.We defined a study to select undiagnosed neurological patients with symptoms of NDDs that overlap with late-onset LSDs.

Material and Methods: Patients enrolled in this study were analysed using a NGS panel including 64 genes involved in LSDs. Familial analyses, enzyme assays and functional studies were used to characterise patients' defects.

Results: In this study, which included 148 patients with NDDS, we identified a patient affected by the late-onset form of Krabbe disease, and 2 patients with pathogenic variants in the lysosomal *GRN* gene, causing frontotemporal dementia, an autosomal dominant disorder.We also identified 11 patients carrying known *GBA* mutations, 3 patients carrying known *GALC* mutations and 3 patients with pathogenic variants in the *ARSA* gene. These 17 patients carried the pathogenic variants at a heterozygous state.

Conclusion: This study emphasizes the importance of including LSDs in the differential diagnosis of NDDs. In addition, compared to the general population, we identified a high percentage of heterozygous carriers of known pathogenic variants involved in LSDs, which suggests that LSD genetic defects may be at a greater risk of developing NDDs.

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P15.149.D Differential coverage of Whole Exome Sequencing commercial kits utilizing DNA derived from diverse tissue sources

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Fuentes[6], Miriam Potrony[2,3,4], Laia Rodriguez-Revenga[2,3,4], Judit Garcia-Villoria[3,4,6], Celia Badenas[2,3,4], Joan Anton Puig-Butillé[1,2,3]

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Background: Whole Exome Sequencing (WES) is gaining increasing relevance both as a molecular biology method and standard diagnostic strategy in medical genetics. However, commercial WES library preparation kits vary significantly in design and coverage. Few studies have assessed the impact of the DNA tissue of origin on WES performance, particularly for rare disease diagnoses where preanalytical factors may compromise DNA quality.

Material and Methods: We evaluated three WES commercial solutions using eight DNA samples from distinct tissue types (four from peripheral blood and four from other sources, including amniotic fluid, postmortem tissue, and skin-derived fibroblasts). A second set of 24 previously sequenced samples (12 peripheral blood and 12 non-blood tissues) was resequenced using the highest-performing kit. A comprehensive bioinformatics pipeline was applied to evaluate key metrics such as coverage uniformity, duplication rates, or estimated library size, among other metrics.

Results: Solution (S1) demonstrated the best overall performance, achieving more uniform coverage, lower duplication rates (8% vs 16–17%), and a larger estimated library size (243M vs 102–129M) compared to S2 or S3. Additionally, S1 achieved 93% of regions covered at 38x (compared to ~80% in S2 and S3). Notably, this kit also consistently delivered superior results for DNA extracted from non-peripheral blood tissues.

Conclusion: This pilot study suggests that solution 1 provides robust performance and is particularly well-suited for samples where DNA quality may be compromised due to preanalytical limitations.

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P15.150.A A case of FINCA syndrome presenting with neurodevelopmental disorder, interstitial lung disease, recurrent infection, and hemolytic anemia: case report and literature review

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Fibrosis, neurodegeneration, and cerebral angiomatosis (FINCA) syndrome is an ultra-rare autosomal recessive disorder, characterized by infantile-onset, progressive cerebropulmonary disease with variable multisystemic symptoms, including immunologic, and hematologic involvement. Biallelic pathogenic variants of the *NHLRC2* gene have been associated with FINCA syndrome. Here, we described the case of FINCA syndrome presenting with neurodevelopmental disorder, interstitial lung disease with subpleural cysts, recurrent infection, and chronic hemolytic anemia. Clinical exome sequencing revealed compound heterozygous variants in *NHLRC2* designated as c.442G>T (p.Asp148Tyr) and c.1220C>T (p.Ala407Val). The variant p.Asp148Tyr has been previously reported in nearly 80% of all patients with this disease. Interestingly, the patients harboring compound heterozygous of variants of p.Asp148Tyr and another truncating variants exhibit more severe than those with p.Asp148Tyr and missense variants. The systemic symptoms include pulmonary disease, recurrent infections, episodic diarrhea, and chronic hemolytic anemia (85.7%, 100%, 71.4%, and 85.7%, respectively in truncating variants and 88.9%, 88.9%, 77.8% and 33.3%, respectively in other missense variants). Patients with the homozygous p.Asp148Tyr trend to have fewer multiorgan involvement compared to those with compound heterozygous of p.Asp148Tyr with other variants (18% of pulmonary disease, 27% of recurrent infection, and none of hemolytic anemia). In conclusion, we described a new FINCA syndrome patient with one common and one novel variants of *NHLRC2*. According to our analysis, certain genotype-phenotype correlation is suggestive in FINCA syndrome although a larger cohort is needed.

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P15.151.B Gene burden testing in a clinical NHS cohort identifies suggestive novel associations with early-onset severe obesity

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Background: Gene-level burden testing of rare variants in sequenced population-based cohorts has identified novel associations with clinically-relevant phenotypes. Routine whole exome sequencing (WES) of NHS patients with rare diseases provides an opportunity to use similar approaches. Genetic testing for young children with severe obesity is commissioned by NHS England through the National Genomic Test Directory and delivered by the NHS East Genomic Laboratory Hub. A virtual panel of 30 genes is applied to WES data; diagnostic yield is low (<4%) suggesting variants in other genes may be involved.

Material and Methods: Gene burden testing was performed exome-wide in a retrospective, case/control, deidentified cohort, using rare (gnomAD AF < 0.1%) and predicted deleterious variants (protein-truncating and missense with REVEL>0.7). Only 11 of 597 cases, referred for early-onset obesity, received a positive diagnosis at the time of clinical reporting (~1.8%); most reported variants were in *MC4R* (n = 8). Controls were patients referred for other indications (n = 3898).

Results: Association with *MC4R* was replicated at p = 0.00030; 4 controls harboured known pathogenic *MC4R* variants, consistent with their observation in the general population. Suggestive novel associations were identified for three genes. Association of these genes with BMI in the UKBiobank cohort and with early-onset obesity in a research cohort is being investigated.

Conclusion: Gene-agnostic statistical approaches can be applied to clinical WES to make scientific discoveries. Key limitations to overcome include limited numbers of rare disease cases in a single testing laboratory, lack of phenotype and genetic

ancestry information, and non-uniform sequencing and data processing steps applied across samples.

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P15.152.C Application of HiFi long-read genome sequencing for the diagnostics of genodermatoses

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Background: Genodermatoses are a complex and heterogeneous group of skin disorders characterized by variable expression and clinical and genetic heterogeneity. To date, a diagnosis can be established in about 50% of cases using DNA-based techniques, such as WES. However, certain genes associated with genodermatoses pose particular challenges for short-read sequencing technologies. For example, the *KRT6A, KRT6B*, and *KRT6C* genes share >95% sequence identity in their coding regions. This high level of homology underscores the challenges in genetic diagnostics. In this study, we evaluate the use of HiFi Revio long-read genome sequencing for diagnostics of genodermatoses.

Material and Methods: Eighteen patients with genodermatoses, who previously had undergone genetic testing using WES or WGS were included the study. PacBio SMRTbell library construction was performed using the SMRTbell Template prep kit, and sequencing was conducted on the PacBio Revio system, with each SMRTbell sequenced on a 25M SMRT cell. Bioinformatic analysis was performed using Genomic Medicine Sweden's Nallo pipeline, and the vcf files were analyzed using in silico gene panels and Alissa Interpret (Agilent).

Results: Validation was performed on Genome in a Bottle (GIAB) and clinical samples with previously known variants. Benchmarking analysis showed high F1-scores for both SNVs (99.92%–99.96%) and INDELs (98.46%–98.77%) in NA12878 and NA24385 GIAB samples. Notably, a pathogenic *KRT6C* variant was identified in a patient with suspected pachyonychia congenita, where prior short-read WGS had been inconclusive.

Conclusion: This study highlights the potential of HiFi Revio long-read genome sequencing for improved diagnostics of genodermatoses.

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P15.153.D Deciphering congenital hemolytic anemia: identifying causal variants in unresolved cases through whole exome sequencing

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Background: Red blood cell (RBC) disorders are a diverse group of hereditary hemolytic anemias (HHA) characterized by variations in clinical presentation and molecular mechanisms. This study aimed to clarify the diagnoses of patients who remained undiagnosed despite continued clinical follow-up.

Material and Methods: Fourteen index cases that remained undiagnosed through clinical evaluation and routine laboratory tests were analyzed using whole exome sequencing (WES). Candidate variations were confirmed, and familial segregation analyses were conducted using Sanger sequencing.

Results: We identified variants explaining the HHA phenotype in 12/14 cases (85%). Variants in *PKLR* gene (n = 4, 28%) were the most commonly encountered, with two cases homozygous and the other two showing compound heterozygosity. Erythrocyte membrane defects were detected in the *SPTB*, *PIEZO1* and *ABCB6* genes in three cases (21%). In one patient, a compound heterozygous *CDIN1* variation was identified in CDA type 1b, while an *ALAS2* variation was found in another patient with sideroblastic anemia. The diagnosis was revised in one case due to a pathogenic homozygous variation detected in the *SLC29A3* gene. Variations in novel candidate genes involved in erythropoiesis were also identified in three cases. In two cases, the genetic cause could not be clearly identified. There was no overlap in causative variants.

Conclusion: HHA exhibits genetic heterogeneity. Diagnoses may be revised, novel candidate genes can be identified through WES, and the potential for compound heterozygosity in known genes should not be overlooked.

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P15.154.A Secondary clinically actionable findings revealed by WES of Bulgarian cohort related to COVID-19 severity

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Background: Analyses involving whole-genome or whole-exome sequencing (WGS/WES) inevitably capture data on the entire genome, often revealing secondary findings (SF) beyond the original scope of investigation. Although the decision to disclose SF resulting from research is still debated, the American College of Medical Genetics and Genomics (ACMG) provides guidelines for reporting SF in clinical exome and genome sequencing (Miller et al., 2023).

Methods: In this study, we examined WES data of a cohort of 444 Bulgarian patients, originally recruited to explore the genetic factors influencing COVID-19 severity. Written Informed consents with opt-in SF reporting for actionable genes were obtained. Variations were found in 71 of the genes and their classification was done according to ACMG recommendations criteria version 3.2.

Results: 67 likely pathogenic (LP) or pathogenic (P) variants were detected in 31 genes (7.55% of all studied variants), found across 61 heterozygous carriers. Of these, 58 variants were unique and 39 were also reported as LP/P in ClinVar. The frequencies of SF in genes linked to cardiovascular diseases, hereditary cancers, and metabolic disorders were 3.38%, 2.14%, and 0.68%, respectively (1.35% in other genes). SF in dominantly/recessively inherited alleles were 65.67%/34.33% respectively. The most frequent SF were identified in *MUTYH* (9 cases, 4 variants), *LDLR* (7 cases, 4 variants), and *FBN1* (5 cases/variants).

Conclusion: Our findings reaffirm that WGS/WES uncovers many clinically actionable secondary findings. As genomic research becomes more affordable, careful ethical consideration of SF disclosure will be essential to maximize both scientific value and individual patient benefit.

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P15.155.B Assessing the diagnostic yield and clinical utility of whole-exome sequencing in undiagnosed pediatric patients: a single-center study from the Czech Republic

Petra Pokorna[1,2], *Katerina Kozelkova*[1], *Eliska Hlouskova*[1], *Sona Adamcova*[1], *Robin Jugas*[1], *Jana Kubatova*[3], *Pavlina Danhofer*[2,4], *Ondrej Horak*[4], *Petr Jabandziev*[2,5], *Tereza Svatonova*[5,6], *Hana Palova*[1], *Dana Knoflickova*[1], *Ondrej Slaby*[1,2,3], *Katerina Slaba*[2,5,6]

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Background: Rare diseases are clinically heterogeneous, primarily genetic conditions affecting fewer than 1 in 2000 individuals. Despite their low individual prevalence, the cumulative impact is substantial, with many patients remaining undiagnosed and undergoing a prolonged diagnostic odyssey. To address this, we integrated whole-exome sequencing (WES) into the diagnostic workflow for undiagnosed pediatric patients at our center, aiming to evaluate its diagnostic yield and actionability.

Material and Methods: Between 2020 and 2024, 101 undiagnosed pediatric patients (44 females, 57 males) were enrolled in a study at the Outpatient Clinic for Undiagnosed Pediatric Patients, Department of Pediatrics, University Hospital Brno, Czech Republic. All patients underwent singleton WES, with selected cases receiving whole-genome sequencing (WGS). Data were bioinformatically processed and analyzed using a phenotype-driven approach.

Results: WES identified or facilitated the discovery of a causal variant in 44% of cases. In two cases, WES suggested a specific phenotype but detected only a heterozygous variant, requiring further WGS analysis to confirm the diagnosis. In another case, WES indicated a homozygous deletion, subsequently validated using MLPA. Among the positive findings, 40 variants were classified as pathogenic or likely pathogenic, including several previously unreported variants. Additionally, four variants initially categorized as VUS were later confirmed as causative through further testing. The overall actionability, enabling at least one type of change in patient care, was 59%.

Conclusion: Our study demonstrates that WES achieves a high diagnostic yield and clinical utility in undiagnosed pediatric patients, reaching the upper range of outcomes reported in published meta-analyses.

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P15.156.C Discrepancy in *CFTR* genotype distribution between cystic fibrosis patient registry and newborn screening data: implications for diagnostic improvement

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Background: *CFTR* mutation distribution varies across populations, impacting cystic fibrosis (CF) diagnosis and treatment eligibility. Ukraine implemented CF newborn screening (NBS) in 2022 as part of a comprehensive screening program for rare diseases. This study compares *CFTR* genotype frequencies between NBS data and the national CF registry, particularly focusing on c.1521_1523delCTT (F508del) allele prevalence, which determines eligibility for elexacaftor-tezacaftor-ivacaftor (ETI) treatment.

Material and Methods: We analyzed *CFTR* variant distribution using the European Cystic Fibrosis Patient Registry (ECFPR) data. The Ukrainian CF-NBS protocol employs immunoreactive trypsin (IRT) measurement followed by DNA testing for 34 common CFTR variants (IRT/IRT/DNA). Next-generation sequencing (NGS) is performed when two pathogenic variants are not initially identified. We compared genotype frequencies between NBS-diagnosed cases and registry data.

Results: The CF registry for 2023 year ($n = 512$) revealed 33% patients were F508del homozygotes, 50% F508del compound heterozygotes, and 17% had other pathogenic variants. The NBS CF-diagnosed cohort showed significantly different distribution ($p = 0.05$): 42% F508del homozygotes, 27% F508del compound heterozygotes, and 31% with other variants. This difference impacts ETI treatment eligibility rates between registry (83%) and NBS (69%) populations.

Conclusion: The discrepancy between registry and NBS genotype distributions suggests potential underdiagnosis of CF patients carrying non-F508del variants, possibly due to milder phenotypes. NBS implementation may provide more comprehensive detection of CF cases across all genotypes.

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P15.157.D Unraveling the genetic basis of recessive neurodevelopmental disorders (NDD) and skeletal dysplasias (SKDs) in 80 consanguineous families through exome sequencing

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Background: Monogenic diseases such as neurodevelopmental disorder (NDD) and skeletal dysplasias (SKDs) inherited as autosomal-recessive (AR) traits are common in populations with a high rate of consanguinity. A higher prevalence of these AR diseases is observed in countries such as Pakistan, where consanguineous marriages are culturally favored, exhibit a higher prevalence of AR diseases. This offers the opportunity to identify new disease-causing genes/mutations associated with AR disorders, helping to uncover their genetic and pathophysiological mechanisms. The aim of the study was to identify new disease-causing genes associated with NDD and SKDs. To achieve this, 80 families with recessive diseases were enrolled and investigated for disease-causing genes.

Material and Methods: Exome sequencing (ES) was performed on 112 individuals from 80 families, primarily presenting with NDD and SKDs. Many of these families had multiple affected individuals.

Results: We were able to identify the underlying genetic cause in 48 families, while 26 remained without a strong candidate variant. Six families required further re-analysis. Overall, a strong candidate variant was detected in about 50% of cases, whereas the remaining 50% lacked a definitive molecular diagnosis. Notably, novel disease-associated gene *NAV3* and candidate genes such as *DOCK1*, *IQCG*, and *HOXC10*, were identified, where biallelic variants were linked to recessive NDD, post axial polydactyly and brachydactyly respectively.

Conclusion: In conclusion, these findings expand the genetic landscape NDD and SKDs by identifying novel disease causing-gene and several candidate genes.

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P15.158.A Mapping the genetic landscape of rare diseases: Insights from Orphanet gene-disease annotations

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Background: Orphanet is a rare diseases knowledge base that includes manually curated annotations of gene-disease relationships. These annotations are derived from scientific publications and follow standardized procedures to ensure accurate linkage decisions.

Material and Methods: Orphanet genetic annotations are linked to rare diseases classified by medical domain and aligned to external genetic resources, including OMIM, HGNC, ensembl and Reactome, allowing the retrieval of gene pathway information. By assessing the distribution of disease-associated genes across medical domains we provide insights into the current state of genetic knowledge on rare diseases. Furthermore, a cross-referencing of the Orphanet gene list with external genetic databases will enable the identification of shared genes and pathways involvement across medical domains.

Results: This work will present the distribution of rare genetic disorders with or without a known molecular cause across medical domains. Orphanet currently includes 4,377 unique genes, of which 4,062 are indexed as disease-causing, with germline mutations being the most common association. Overall, 3,250 out of the 4,659 genetic disorders present in Orphanet have at least one known gene disease-association. Rare developmental defects during embryogenesis and rare neurological diseases represent the categories with the highest genetic disease's distribution. Data normalization will also show the average number of genes per disease by medical specialty. Leveraging Orphanet's gene mappings to external databases will allow further exploration of rare disease genetics.

Conclusion: The Orphanet gene-disease annotations combined with disease classification by medical domain and mappings to other gene databases provide a powerful resource for exploring the genetic landscape of rare diseases.

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P15.159.B *PLEC* gene variants: from biallelic mutations in cholestasis to first evidence of adult-onset disease in heterozygous carriers

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Background: Cholestatic jaundice in infants and adults represents a significant clinical challenge, potentially leading to severe outcomes without prompt treatment. PLEC, encoding plectin a cytoskeletal linker for intermediate filaments - has recently emerged as a candidate gene in cholestasis. While initially identified in siblings with progressive familial intrahepatic cholestasis, PLEC's role in adult cholestasis remains largely unexplored.

Material and Methods: Liver biopsy samples from patients with cholestasis carrying PLEC mutations underwent detailed immunohistochemical and immunofluorescence analysis to assess protein expression patterns and subcellular localization.

Results: Our study identified three distinct cases with PLEC variants: A young woman presenting with severe cholestasis since adolescence, carrying compound heterozygous missense variants, demonstrated significant alterations in both plectin and bile salt export pump protein expression; An adult patient with two in cis heterozygous missense variants exhibited a milder cholestatic phenotype, with immunohistochemical analysis revealing correspondingly less severe protein alterations; A 17-year-old male with obstructive cholestasis harbored a previously unreported heterozygous missense variant.

Conclusion: Our findings provide strong evidence that PLEC mutations may be associated with familial intrahepatic cholestasis phenotype. Immunofluorescence studies offer crucial insights into the molecular mechanisms underlying cholestasis development in

these patients. These newly identified PLEC mutations expand our understanding of cholestasis genetics and highlight the significant phenotypic variability between pediatric and adult-onset presentations, suggesting age-dependent penetrance or modifier effects. Like other PFIC genes where heterozygous mutations result in milder phenotypes, PLEC mutations present on a single allele have been associated with transient episodes of cholestasis.

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P15.160.C Diagnostic role of whole exome sequencing in children with medical complexity

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The aim of this study was to analyse the diagnostic yied of Whole Exome Sequencing (WES) in a cohort of 140 children with medical complexity, and to evaluate the predictive value of specific symptoms and clinical signs. WES identified a pathogenetic or probably pathogenic variant (C5-C4) in 84/140 patients (60%), a variant of uncertain significance (C3) in 37/140 patients (26.4%) and a benign or potentially benign (C2-C1) variant in 19/140 patients (13.6%). Medical history, clinical phenotype and imaging studies (brain, heart, abdomen) were analysed for all patients: consanguinity of the parents had a positive predictive value (PPV) of 78.6%, onset of symptoms <1 year a PPV of 60.4%, clinical signs of regression a PPV of 68.2%, pathological brain magnetic imaging a PPV of 62.5%, pathological echocardiography a PPV of 60.7%, pathological nephro-urologic ultrasound a PPV of 45.4%, pathological ophthalmological examination a PPV of 70.9%, hypotonia or hypertonia a PPV of 68.4% and microcephaly or macrocephaly a PPV of 71.8%. In conclusion, WES led to a rapid, accurate diagnosis in 60% of cases. Consanguineity of the parents increased the diagnostic yields, as expected; furthermore, hypo/hypertonia and micro/macrocephaly emerged as key clinical signs. Further research is warranted to validate these data.

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P15.161.D Adult-onset β-thalassemia major is an acquired imprinting disorder

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Background: Adult-onset β-thalassemia major is an extremely rare disorder observed in β-thalassemia carriers, resulting from chromosome 11p15 somatic loss of heterozygosity. We present the case of a man, carrier of the HBB:c.118C>T β^0 globin mutation, who developed progressive anemia, characterized by increased hemolysis, elevated HbF levels (39.8%), and diffuse hematopoietic hyperplasia. Over the years his Hb levels gradually declined from 12 g/dL to 8 g/dL, prompting further evaluations.

Material and Methods: Acquired *HBB* mutation homozygosity was observed in peripheral leukocytes, while heterozygosis was confirmed on a buccal swab. SNP-array on bone marrow DNA revealed a 14.7 Mb pUPD on 11p15.2-11p15.5 (11p15.2-11p15.5, 219089_14922363), explaining the homozygosity for the β-globin mutation. Exome sequencing on peripheral leukocytes and total RNA-seq on bone marrow were performed to explore the biological mechanisms driving pUPD 11p clonal expansion.

Results: RNA-seq demonstrated loss of expression of the *HBB* gene and overexpression of the *IGF2*, *HBG1*, *HBG2*, and *BGLT3* genes. No known HPFH-associated DNA variants were identified.

Conclusion: The propositus inherited the β^0-globin mutation from his father and hematopoietic stem cells developed adult-onset pUPD 11p causing β^0 homozygosity. As observed in Beckwith-Wiedemann syndrome, this pUPD duplicated the paternally inherited active allele of the imprinted *IGF2* gene. RNA-seq confirmed *IGF2* overexpression, which is likely driving the clonal expansion of β^0-homozygous cells. Notably, overexpression of *HBG1* and *HBG2*, along with high levels of HbF, may be explained by increased expression of the lncRNA BGLT3. The molecular mechanisms leading to BGLT3 activation are unknown but presumably related to the UPD event.

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P15.163.B Genotype-phenotype correlations in the analysis of *GFAP* Variants in Type I Alexander Disease subtypes

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Background: Alexander disease (AxD) is a leukodystrophy caused by autosomal dominant gain-of-function mutations in the Glial Fibrillary Acidic Protein (GFAP) gene. AxD presents as Type I AxD and Type II AxD, based on clinical and neuroradiological features. The variability in Type I disease progression led to a subclassification into Ia, Ib, Ic, and Id, and intermediate Ic/Id. This study aimed to deepen the understanding of genotype-phenotype correlations in Type I AxD subtypes

Material and Methods: *GFAP* variants have been deeply analyzed in a cohort of 74 genetically confirmed Type I AxD patients divided in Ia-Ib-Ic-Ic/d-d. Analyses have been performed within each subgroup to identify elements differently stratifying the five groups and, in addition, focusing on the two larger sets based on ability (Ic+Ic/d+Id) or not (Ia+Ib) to acquire autonomous ambulation

Results: 1.progressive decrease in *GFAP* allelic heterogeneity from Ia to Id2.opposite extent of variants detected in exon 1 and in exon 4, directly and inversely correlating with Ia to Id stratification, respectively. 3. p.R239 and p.R79 never detected in Id and Ia, respectively 4.Different p.R79 changes stratify patients with/without autonomous ambulation. 5.CADD scores showed an

inverse correlation with autonomous ambulation 6.Median age of onset: progressive increase from Ia to Id

Conclusion: These results suggest that specific GFAP variants could predict disease progression in Type I AxD, suggesting a refinement of classification and improving patient outcomes. These findings underscore the importance of both genetic and clinical factors in the management of AxD, particularly as therapeutic interventions are being developed

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P15.164.C Genetic modifiers influence: A novel *COL4A4* variant delays kidney failure in a homozygous Alport syndrome patient and expedites kidney failure in a heterozygous patient

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Background: Patients who are heterozygous for *COL4A3* or *COL4A4* pathogenic variants develop autosomal dominant Alport syndrome (ADAS) generally of milder phenotype than patients with biallelic mutations. A common feature is familial microscopic hematuria (FMH), which may or may not progress to added proteinuria and chronic kidney disease (CKD).

Material and Methods: We studied clinically and genetically nine patients from four families that segregate FMH, proteinuria, and CKD, with clinical suspicion for Alport syndrome.

Results: Molecular and genetic testing revealed the inheritance of a common *COL4A4* variant, substituting arginine for glycine-1248, thus disturbing the protein triple-helical domain (COL4A4:c.3742G>C(p.Gly1248Arg). A homozygous patient had the Alport phenotype, including hearing impairment and ocular findings, but a much later age-at-onset of kidney failure (46 years), compared to the age usually seen in patients with other mutations. Importantly, a relative patient, heterozygous for the variant developed kidney failure at 58 years, without extrarenal findings.

Conclusion: The delayed kidney failure of the homozygous patient could be attributed to the position of the substitution near a natural interruption of the α4-chain collagenous domain. We hypothesize that the disruption conferred during propagation of the collagen-IV triple-helix is less drastic, affecting less severely the protomer configuration, and maintaining residual activity for network formation in the glomerular basement membrane. The heterozygous patient co-inherited a *LAMA5* gene variant that probably acted as a genetic modifier negatively influencing the phenotype. The findings support the notion that collagen-IV nephropathies constitute an Alport spectrum condition with diverse manifestations.

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P15.165.D Investigating*TNNC1*gene inheritance and clinical outcomes through a comprehensive familial study

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Background: Hypertrophic cardiomyopathy (HCM) and restrictive cardiomyopathy (RCM) have significant phenotypic overlap and a similar genetic background. This study focuses on the genetic testing of two interconnected families with four infants diagnosed with fatal RCM associated with bi-ventricular hypertrophy.

Material and Methods: Biological samples of the deceased infants were analyzed with exome sequencing, while direct ancestors and siblings of the deceased infants were analyzed using the Ventrilia Cardiovascular NGS test (Medicover Genetics), that analyzes 292 genes that can cause multiple cardiovascular conditions with complex phenotypes. The rest of the family members were tested using Sanger sequencing.

Results: A pedigree spanning six generations was created, involving 56 individuals participating in this study. Our genetic analysis revealed that homozygosity for NM_003280.3(*TNNC1*):c.23C>T(p.Ala8Val) results in HCM which evolves into a severe RCM phenotype, leading to fatal outcomes during infancy. Identified heterozygous individuals were predominantly asymptomatic or exhibited later onset of HCM, inherited in an autosomal dominant manner.

Conclusion: Our study provides strong evidence that the homozygous expression of A8V TnC exacerbates disease progression, leading to the transition from HCM to RCM, ultimately culminating in death. This indicates that variants in the TNNC1 gene associated with HCM can also lead to severe RCM in infancy, showing different inheritance patterns. Overall, this study underscores the complex nature of genetic inheritance in cardiomyopathies and emphasizes the vital role of genetic testing in providing essential insights crucial for diagnosis, prognosis, early intervention, and the development of potential treatment strategies.

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P15.166.A Clinical and Molecular Heterogeneous Pathway Disorders: RASopathy Syndromes

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Background / Objective: RASopathies are a group of multisystem disorders caused by genetic variations that disrupt the RAS/MAPK signaling pathway. These conditions are classified into four main groups: Noonan syndrome (NS) and related disorders, Neurofibromatosis type 1, Non-systemic RASopathies, and Mosaic RASopathies. This study aims to evaluate the clinical and molecular characteristics of patients diagnosed with RASopathies.

Method: Clinical and molecular data of 16 patients diagnosed with RASopathies were retrospectively analyzed at the Department of Pediatric Genetic Diseases, Ege University.

Results: Of the cases diagnosed with RASopathies, 8 are female and 8 are male. Pathogenic or likely pathogenic variants were detected in 93.7% (n = 15) of the cases. Variants in genes related to Noonan syndrome and related disorders (PTPN11 in 5 cases, BRAF in 2 cases, SHOC2 in 1 case, SOS1 in 1 case, KRAS in 1 case) were detected in 10 patients. Craniofacial dysmorphic features were present in all Noonan syndrome cases. In 4 patients with non-systemic RASopathies, three variants were found in the RASA1 gene, two of which were novel. One patient with an NF1 variant was diagnosed with NF-NS based on clinical and molecular findings, and Selumetinib treatment was planned due to extensive plexiform neurofibromas. Another patient with isolated mosaic RASopathy underwent DNA isolation from a skin biopsy, revealing no variants in KRAS, NRAS, or HRAS genes.

Conclusion: This study emphasizes the clinical and molecular heterogeneity of RASopathies. Next-generation sequencing methods have increased diagnostic rates, contributing to genetic counseling, prognosis prediction, understanding pathophysiology, and developing therapeutic approaches.

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P15.167.B Genetic variants related to childhood neurodevelopmental disorders in well-characterized Finnish cohort

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Background: Pediatric neuropsychiatric disorders like Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) have long-term impacts on individuals, families, and the healthcare system. Genomic studies have identified various common, rare, and de novo variants linked to these disorders, raising questions about the role of genetic testing in clinical practice. While genetic testing is not currently used to guide ASD intervention, understanding genetic factors can help families better comprehend the disorder. This study aims to evaluate the clinical significance of genetic variants in a Finnish cohort and identify patterns that predict the presence of clinically relevant variants.

Material and Methods: Twenty-one children (ages 5–8) with complex neurodevelopmental phenotypes were recruited from the Pediatric Neuropsychiatric Unit of Helsinki University Hospital. A multidisciplinary team performed comprehensive clinical assessments, and whole genome sequencing (WGS) was conducted on both patients and their parents. Our data analysis pipeline integrates multiple tools for variant calling, interpretation, and curation, with targeted sequencing to validate relevant findings.

Results: Preliminary findings suggest that most children carry potentially high-impact variants linked to neurodevelopmental disorders, including ASD. This contrasts with prior clinical CMA results, where only four out of twelve children had genetic findings. These results require validation.

Conclusion: WGS identifies potentially clinically relevant variants at a much higher rate than current clinical methods. This suggests that integrating WGS into routine evaluations could lead to more precise, personalized diagnoses for children with complex neurodevelopmental symptoms.

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P15.168.C WES analysis of CN-LOH regions increases diagnostic yield in patients with neurodevelopmental disorders and/or congenital anomalies

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Background: Regions with Copy-Neutral Loss of Heterozygosity (CN-LOH) occur in human genomes because of parental consanguinity, recombination events or other chromosomal rearrangements, and lead to obligatory homozygosity for variants in autosomal recessive genes. Whole Exome Sequencing (WES) analysis, targeting CN-LOH regions identified by SNP array, can enhance genetic diagnosis through the detection of homozygous pathogenic variants.

Material and Methods: 7 patients with neurodevelopmental disorders and/or congenital anomalies, with extensive regions of CN-LOH were included in the study. Genomic DNA was extracted from peripheral blood samples using the QiaSymphony system (Qiagen, Germany). CMA was performed with the G3 4 × 180K CGH+SNP microarray platform (Agilent Technologies, USA), that contains ~60.000 SNP probes, providing detection of CN-LOH regions larger than 5 Mb across the genome. Consequently, WES was performed with the Human Exome core 2.0 library preparation kit (Twist Biosciences, USA) on a NovaSeq 6000 instrument (Illumina, USA). Alignment and variant calling were performed with GATK (Broad Institute, USA). Variant annotation and interpretation were performed with Franklin (https://franklin.genoox.com).

Results: On average 69 Mb of CN-LOH regions were identified per sample, ranging from 15 Mb to 116 Mb. WES analysis of these regions detected novel homozygous causative variants in 2/7 cases (28%). Specifically, the NM_138701.4:c.238del variant in *MPLKIP*, associated with Trichothiodystrophy 4 (#234050) and the NM_016194.4:c.819C>A variant in *GNB5*, associated with Lodder-Merla syndrome 1 (#617173) were identified in patients 1 and 4, respectively.

Conclusion: WES analysis targeted at CN-LOH regions can enhance the identification of novel rare recessive pathogenic variants and increase diagnostic yield.

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P15.169.D Revisiting cold cases: a single center's experience with clinical and bioinformatic re-evaluation of undiagnosed

disorders

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Background: Despite the growing availability and improved capabilities of genetic testing, some patients remain without a molecular diagnosis, even when physical signs and symptoms strongly suggest an underlying hereditary disorder. We present our experience with cases referred to the Undiagnosed Clinic.

Material and Methods: Cases were discussed by a team of clinical geneticists, including refinement of the clinical phenotype and consideration of potential diagnoses. Reevaluation of exome (ES) or genome sequencing (GS) data was done by the team. Additional clinical or research tests were done as recommended.

Results: 81 cases were evaluated over a period of 40 months. The most common reason for referral was intellectual disability/developmental delay with dysmorphism or additional manifestations (42/81, 52%). A new diagnosis was achieved in eight (10%) cases, including four cases where reanalysis revealed a novel or recently discovered gene, and one deletion that included a novel gene. One variant was identified that had previously been filtered out due to poor sequencing quality. In one case with negative CMA, ES and GS, a karyotype was done revealing a de novo balanced translocation t(X;13) not detectable by the previous methods. In addition, at least four candidate variants were identified in the cohort. In three cases it was decided that previously-detected variants were likely causative.

Conclusion: Clinical reevaluation and reconsideration of the diagnostic approach as well as ES/GS reanalysis were useful in diagnosing cases with a high suspicion for a monogenic etiology. The overall yield is potentially higher given that additional results of tests are pending.

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P15.170.A Genome sequencing in routine clinical care: The good, the bad, and the uglY

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Background: Genome sequencing (GS), which allows for comprehensive genome analysis, is increasingly part of routine clinical care in rare diseases. It not only leads to new diagnoses but also uncovers pitfalls in structural variant (SV) detection, and challenges with interpreting Y-chromosomal variants.

Material and Methods: Twelve illustrative cases were selected from routine short-read GS gene panel analyses.

Results: GS finally revealed pathogenic variants for clinically diagnosed patients: a large insertion-deletion of an inverted segment from Xp22.33 affecting the *MECP2* gene on Xq28, confirming Rett syndrome; a de novo deletion including the first two exons from the *KANSL1* gene, causing Koolen-de Vries syndrome; and an intragenic deletion in the *EHMT1* gene, justifying the episignature for Kleefstra syndrome. GS also detected cytogenetic abnormalities: a mosaic ring chromosome 18 was identified and confirmed via karyotyping, while an unbalanced translocation, der(20)t(16;20)(q23.3;q13.33) -below the resolution of karyotyping- was confirmed with FISH. Interestingly, in two other cases, subsequent karyotyping did not confirm abnormal findings: a possible reciprocal translocation t(1;22)(p13.3;q12.2) and a suspected translocation t(1;7)(p31.3;p14.3). Additionally, a reciprocal translocation t(8;14)(p23.1;q32.1) and a mosaic 10qter deletion were detected, albeit after extensive inspection. GS data visualization and interpretation of the Y chromosome can be hampered by forced read-alignment of the pseudoautosomal regions to the X chromosome. Nonetheless, with careful inspection and handling of GS data, aberrant Y chromosomes were detected in three cases.

Conclusion: GS enables the diagnosis of genetically unsolved, clinically diagnosed patients. While GS can detect (complex) SVs, interpretation requires caution and cytogenetic expertise.

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P15.172.C A hypomorphic *FLVCR2* variant resulting in moderate transport deficiency causes hydranencephaly syndrome with brain calcifications

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Background: FLVCR2, a conserved member of the major facilitator superfamily (MFS), transports small molecules across lipid bilayers. Loss of the murine ortholog Mfsd7c leads to brain angiogenic deficiency and lethality. Recessive *FLVCR2* variants cause Fowler syndrome (PVHH), a severe condition with microcephaly, skeletal deformities, and cerebrovascular defects. Despite reported cases, evidence of FLVCR2 variant pathogenicity remains limited.

Material and Methods: We investigated a fetal case of Fowler syndrome using exome sequencing, identifying compound heterozygous FLVCR2 variants: maternal c.1124+3_1124+6del and paternal p.(Arg492Trp). Minigene assay assessed mRNA splicing effects. Protein modeling (Rosetta, DynaMut2) assessed

the structural effect of the p.(Arg492Trp) variant. Radiolabeled-choline and ethanolamine transport assays in HEK 293 cells measured FLVCR2 transport activity.

Results: The c.1124+3_1124+6del variant impaired FLVCR2 exon 5 splicing. The p.(Arg492Trp) substitution caused a 50–60% reduction in FLVCR2 transport activity, resulting in a net activity of 25–30%. These findings indicate that the p.(Arg492Trp) variant significantly impacts FLVCR2 function. Protein modeling confirmed the destabilizing effects of the substitution, while transport assays established a functional deficiency of the FLVCR2 protein.

Conclusion: Our findings suggest that FLVCR2 deficiency may be sufficient to cause PVHH, even without a complete loss of transport activity. These results highlight the potential involvement of extragenetic factors in the pathophysiology of this complex condition, advancing our understanding of Fowler syndrome and FLVCR2-related pathologies.

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P15.173.D Transcriptome profiling of patients with brain arteriovenous malformations

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Background: Arteriovenous malformations (AVMs) of the brain are pathological tangles of blood vessels where arteries connect directly to veins without intermediate capillaries, leading to the formation of a cluster of abnormal vessels. RNA sequencing enables the identification of genes potentially involved in the mechanism of AVM development.

Material and Methods: The study was conducted on a cohort of three patients from Kazakhstan with brain AVM. RNA extracted from tissue by using RNAqueous™-4PCR Total RNA Isolation Kit. RNA sequencing was performed on the DNBSEQ platform). with an average yield of 8.51G data per sample. The analysis of the obtained data was carried out using the Dr.Tom systems support program (https://www.bgi.com/global/service/dr-tom. To validate the transcriptome data by qRT-PCR assays.

Results: The average alignment ratio of the sample comparison genome was 97.68%, which was comparative. The average alignment of the gene set was 53.76%; a total of 123252 genes were detected. Venn diagram indicates that 8028 DEGs were common to all samples. The bioinformatics analysis showed the biological processes and pathways related to angiogenesis, cell adhesion, signal transduction and signaling molecules and interaction were significantly enriched.

Conclusion: The integration of genomics and transcriptomics data broadens our understanding of key genetic mutations, signaling pathways, and molecular mechanisms involved in the pathogenesis of AVMs.

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P15.174.A Whole genome sequencing in unsolved cases of Russian patients affected with inherited retinal diseases

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Background: Autosomal recessive forms account for more than 55% of all inherited retinal diseases (IRDs). Whole genome sequencing (WGS) offers significant advantages over targeted gene panels.

Methods: We performed WGS on 56 unrelated Russian patients in whom a single pathogenic/likely pathogenic variant in the *ABCA4, CNGB3, USH2A, CRB1, EYS, CNGA3*, and *CEP290* genes had been previously identified by panel sequencing (212 genes).

Results: In 30 out of 56 patients (53.57%) the cause of the disease could be identified. Among them, a pathogenic variant on the second allele in the studied genes was detected in 17 patients (56.67%). In the *EYS* gene in five patients the following alterations were detected: large deletion partially involving exon 19 (2 patients), deletions of exon 1 and exons 3–5, inversion in the 6q12 region with a breakpoint in the 13th intron of the gene. In the *ABCA4* two previously described intronic variants c.4539+2064C>T and c.6817-679C>A were identified in two patients, and one patient had a deletion of exon 30. In the *CEP290* a previously undescribed intronic variant c.2818-955A>G (spliceAI, z-score 0.99) was identified in one patient. Large deletions of exon 4 and exons 22–24 were detected in the *USH2A* gene in two individuals. The remaining 13 patients had variants in other genes: *CWC27, CFAP410, TRPM1, TTLL5, NR2E3, CNGB1, BBS5* and *ALMS1*.

Conclusion: This study emphasized the utility of WGS in the diagnosis of unresolved cases of IRDs.

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P15.175.B Primary care implementation of a genetic test for MODY2 screening

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Background: Maturity Onset Diabetes of the Young 2 (MODY2) presents with mild hyperglycemia devoid of microvascular (and possibly macrovascular) complications, often mistaken for other diabetes types. Access in primary care genetic testing for MODY2 is a major challenge but needed to realign treatment with the clinical recommendation which consists mainly of lifestyle modification alone. The objective is to evaluate the implementation of a validated genetic test for MODY2 in primary care.

Material and Methods: A developmental evaluation with qualitative and quantitative data sources is being carried out. This evaluation is supported by the RE-AIM model, a conceptual framework to guide our research into patients' and healthcare professionals' experiences of using the MODY2 screening test. Asynchronous online training on MODY2 and genetic testing, with

pre/post questionnaires, is provided to healthcare professionals (HCPs). HCPs propose the study to patients and, with consent, provide them a buccal cell collection kit. To date, 54 HCPs are participating in the project and 70 patients have been collected, including 13 positive for MODY2.

Results: Conditions for the implementation of the genetic test as well as the effects of training on the acquisition of knowledge by HCPs will be presented. This study also document the implementation process of a genetic test in a primary care setting and the essential factors to be considered for widespread deployment in other Canadian primary care clinics.

Conclusion: Evaluation of the implementation will identify facilitating and limiting factors, with the aim of establishing implementation standards for a provincial scaling-up strategy as a next step.

C.O.I. Disclosure:

K. Tremblay: None. **M. Poitras:** None. **J. Leblanc:** None. **P. Caron:** None. **A. Fournier:** None. **J. Bouchard:** None. **L. Girard:** None. **E. Boivin:** None. **V.T. Vaillancourt:** None. **C. Savard:** None. **N. Bettez:** None. **L. Bouchard:** None. **S. Legeay:** None.

P15.176.C A novel homozygous splice-site variant in *WDR4* results in rare microcephaly syndrome

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Background: Homozygous/compound heterozygous pathogenic variants in the *WDR4* gene are linked to Microcephaly, growth deficiency, seizures, and brain malformations syndrome (MIM:618346) or Galloway-Mowat 6 (MIM:618347) syndrome. This study identifies a novel homozygous splice-site variant in *WDR4* in two siblings. To date, only 9 patients with 8 pathogenic *WDR4* variants are described, of which only one is a variant in the canonical splicing site.

Material and Methods: Clinical evaluations and pre-test counseling were conducted. Whole exome sequencing (WES) was performed, followed by segregation analysis in family members.

Results: Two siblings exhibited intrauterine growth delay, poor postnatal growth, delayed motor and intellectual development, and minor dysmorphic features. WES identified a novel homozygous duplication at the +2 position of the exon 10 donor splice site in *WDR4* (NM_018669.6:c.1045+2dup). Segregation analysis confirmed the variant in the affected siblings, with parents and one unaffected sibling being heterozygous, and another unaffected sibling homozygous for the normal allele. SpliceAI predicted a donor site loss (Δ score = 0.96), classifying the variant as likely pathogenic. RNA-sequencing is planned to validate splicing impact. The previously described canonical splice-site variant shows only partial phenotypic overlap, with varying severity and clinical manifestations among affected family members. While 3 of 4 patients in the described family had glomerulosclerosis, our patients currently show no renal involvement. Neurologic manifestations also differ in features and severity between the described cases and our patients.

Conclusion: This case contributes to the limited literature on *WDR4* variants, expanding knowledge about phenotypic heterogeneity and the potential for diverse clinical presentations.

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P. Sviridov: None. **A. Voskanyan:** None.

P15.177.D Investigation of HS3ST6 mutations in hereditary angioedema

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Background: Hereditary angioedema (HAE) is a rare autosomal dominant disorder characterized by recurrent episodes of subcutaneous and submucosal edema, which can be life-threatening when affecting the airways. HAE types 1 and 2 are caused by mutations in the C1-inhibitor gene, while type 3 is linked to mutations in Factor XII. Additional genes, including PLG, ANGPT1, KNG1, MYOF, and DAB1P, have been associated with unknown HAE (U-HAE). Bork et al in 2021 identified a novel disease-linked mutation for HAE in **HS3ST6** gene, the **c.430A>**T p.(Thr144Ser). This study aimed to investigate a cohort of 160 U-HAE patients, in whom no mutations were found in other HAE-associated genes, to detect this or other HS3ST6 mutations.

Material and Methods: 160 probands diagnosed with HAE, previously studied with NGS custom panel HaloPlex HS Target Enrichment System (Agilent) for genes ANGPT1 (NM_001146.5), SERPING1 (NM_00006.2), PLG (NM_000301.4), MYOF (NM_013451.4), KNG1 (NM_001102416.3), and F12 (NM_000505.3), were examined via Sanger sequencing for the HS3ST6 gene.

Results: The c.430A>T p.(Thr144Ser) mutation was not detected in any patient. However, three novel variants were found: c.488C>T p.(Ala163Val) (in 65 patients, in 7 in a homozygous condition), c.574G>A p.(Ala192Thr) (in 63 patients, in 12 in homozygous condition) and c.946C>G p.(Leu316Val) (in 2 patients in heterozygous condition)

Conclusion: Although HS3ST6 remains a candidate gene for U-HAE, the identified variants were classified as benign by in silico analysis. Only the c.946C>G p.(Leu316Val) is reported in the database ClinVar as a variant of uncertain significance (VUS). Further investigations with an expanded cohort are necessary.

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P15.178.A Identification and genetic analysis of novel mutations in patients with branchial anomalies

Klaudia Cipkova[1], *Marek Sklenar*[1], *Lukas Varga*[1,2], *Miloslav Karhanek*[1], *Miroslav Sabo*[1], *Martina Skopkova*[1], *Lenka Radicova*[3], *Milan Profant*[2], *Daniela Gasperikova*[1], *Silvia Borecka*[1]

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Background: Branchial cleft anomalies are congenital anomalies that develop from the first to the fourth pharyngeal clefts. They can manifest as preauricular sinuses, cervical cysts, or fistulae and present a diagnostic feature of syndromic diseases, such as Branchio-oto-renal (BOR) syndrome, Branchio-otic (BO) syndrome and Oculo-auriculo-vertebral spectrum (OAVS). The aim of the study was identification and genetic analysis of novel mutations in patients with branchial anomalies.

Material and Methods: Eleven families diagnosed with branchial anomalies were included in this study. Samples were

analyzed by WES, WGS, CNV, MLPA and Sanger sequencing. The minigene assay and Nanopore sequencing of nasopharyngeal swab samples was performed to confirm the potential pathogenic effect of splice site variants.

Results: One novel variant of unknown significance in the *SIX1* gene and a novel dominant pathogenic frameshift variant in the *PAX1* gene were identified in two families. Two heterozygous whole *EYA1* gene deletions and four pathogenic variants in the gene *EYA1* were identified in six families. One novel likely pathogenic variant *EYA1*:c.318G>A, one novel deletion (*EYA1*:c.1051-80_1052del) and a pathogenic variant *EYA1*:c.1360+5G>A were identified in three families. Using minigene assay, we confirmed the shortening or skipping of the affected exons. The subsequent cDNA analysis from nasopharyngeal swabs clarified the results of the minigene assay.

Conclusion: Branchial anomalies are mostly caused by pathogenic variants in the *EYA1* gene, which contribute to the BOR syndrome. Nasopharyngeal swabs are a suitable, easily available material to examine the transcriptional profile of the *EYA1* gene in vivo.

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P15.181.D Case studies of diagnostic whole exome sequencing analysis using Franklin Genoox

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Background: We perform diagnostic exome assessment using Franklin Genoox for secondary and tertiary analysis. The combination of the broad range of exome sequencing with Franklin's ability to select relevant variants based on phenotype defined by HPO terms allows for streamlined diagnostic exome assessment.

Material and Methods: We sequence the whole exome using Twist Bioscience panel (Exome 2.0 VCGS) and NovaSeq X Plus (Illumina). Primary analysis is done using DRAGEN software (FPGAtechnology), and secondary and tertiary analysis is performed using Franklin (Genoox). The assessment process focuses on genes associated with the phenotype as defined by HPO terms. We evaluate both SNP and CNV variants. Additionally, we report secondary findings as defined by ACMG recommendations, as well as pathogenic variants in ten most common recessive diseases in our population. Franklin integrates publicly available data from population, disease and sequence databases and published literature. Franklin also uses machine learning to select relevant genetic variants and suggest automated classification based on ACMG classification criteria.

Results: We analyzed 276 whole exome cases, including 72 prenatal and 204 postnatal cases. The majority of the prenatal cases were trio analyses, indicated due to increased nuchal translucency or congenital developmental defects. We identified the genetic cause in 14% of prenatal and 24% of postnatal cases.

Conclusion: The success of finding a causal variant is lower in prenatal cases than in postnatal cases, likely because the phenotype of postnatal cases can be described more specifically. We would like to present some interesting case studies from our diagnostic exome analysis.

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P15.182.A Summary of 14-year single-center experience in fragile X syndrome diagnostics in Poland: Is a dedicated test still necessary?

Urszula Zawadzka-Więch, *Danuta Sielska-Rotblum, Marlena Młynek, Sylwia Purwin, Agata Cieślikowska, Dorota Wicher, Monika Kowalczyk, Justyna Pietrasik, Piotr Iwanowski, Agata Skórka, Katarzyna Iwanicka-Pronicka, Maria Jędrzejowska, Agnieszka Madej-Pilarczyk*

Children's Memorial Health Institute, Warsaw, Poland.

Background: Fragile X syndrome (FXS) is one of the most common monogenic causes of intellectual disability (ID) and autism spectrum disorders (ASD), affecting approximately 1 in 4,000 males and 1 in 8,000 females worldwide. Over 99% of FXS cases are due to the expansion of CGG trinucleotide repeats in the *FMR1* gene located on the X chromosome. This study presents a summary of our 14-year experience in FXS diagnostics.

Material and Methods: From 2011 to 2024, 1467 patients with a neurodevelopmental phenotype suggestive of FXS were referred for genetic testing. Until 2023, molecular data from 1260 patients was analysed using a PCR-based screening test with GeneScan and/or Methylation-sensitive Multiplex Ligation Probe Amplification (MS-MLPA). In 2024, 207 patients were tested using screening, MS-MLPA and/or the newly introduced Tripled-primed PCR (AmplideX PCR/CE FMR1 Kit) method.

Results: Between 2011 and 2023, the full mutation in the *FMR1* gene was detected in 17 of 1260 (1.3%) probands. A total of 171 patients (13.6%) did not yield conclusive results. In 2024, among 207 patients, 7 (3.4%) were found to have the full mutation. Family testing identified the full mutation in 1 patient and a mosaic of full mutation/premutation in 2 others.

Conclusion: Complete FXS diagnostics increases the detection rate of FXS and enables carrier status analysis in the patient's relatives. In the era of high-throughput molecular diagnostic methods, such as aCGH/NGS, we recommend initial FXS testing in patients with ID/ASD phenotype, as it can significantly shorten the diagnostic process while being cost-effective.

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P15.183.B Combined RNA and genome sequencing identifies a novel homozygous intronic variant in *SNX14* in an individual with autosomal-recessive Spinocerebellar Ataxia (SCAR20)

Melanie Brugger[1,2], *Alice Saparov*[1,3,4], *Diana Ballhausen*[5], *Kevork Derderian*[1], *Ivana Dzinovic*[1,4], *Robert Kopajtich*[1,6], *Elisabeth Graf*[1,2], *Bavarian Genomes Network for Rare Disorders*[7], *Thomas Meitinger*[8,2], *Matias Wagner*[1,2,4], *Holger Prokisch*[9,2,4], *Juliane Winkelmann*[1,4,10], *Michael Zech*[1,4,11]

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Germany, [11]Institute for Advanced Study, Technical University of Munich, Garching, Germany.

Background: Performing whole genome sequencing (WGS) in individuals with rare disorders usually leads to the identification of numerous rare or previously undescribed non-coding variants in disease-associated genes. Unbiased analysis of RNA sequencing data can be a vital tool in variant interpretation when performing WGS to increase the diagnostic yield in previously undiagnosed patients.

Material and Methods: We performed short-read WGS of a patient and her healthy parents and RNA sequencing of a patient-derived fibroblast cell line in the frameworks of the research project "Bavarian Genomes". Transcriptomic data was analysed for expression and splicing outliers with OUTRIDER and FRASER tools.

Results: Expression and splicing outlier analysis revealed a significantly reduced RNA expression of Sorting Nexin 14 (*SNX14*; fold change: 0,21; p(adj) = 6,82 × 10-57) in an index case born of consanguineous parents presenting with global muscular hypotonia, developmental delay, movement disorder and cerebellar atrophy. Parallel analysis of WGS data lead to the prioritization of a homozygous intronic variant in intron 12 of *SNX14* (NM_001297614.2:c.1108+28A>G). Transcriptomic data showed an integration of 28 base pairs of intronic sequence into *SNX14* mRNA disrupting the reading frame. This led to the diagnosis of autosomal recessive Spinocerebellar Ataxia 20 (SCAR20) in the index.

Conclusion: By combining expression outliers from transcriptomic data with short read WGS data, we could identify a novel pathogenic intronic variant in *SNX14* as the cause of SCAR20. This example illustrates the benefit of parallel RNA and WGS sequencing in individuals with previously undiagnosed neurodevelopmental disorders, especially in the context of consanguinity.

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P15.184.C Exome sequencing enhances diagnosis of hereditary epidermolysis bullosa in tunisian patients

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Background: Hereditary epidermolysis bullosa (EB) comprises a heterogeneous group of genodermatoses characterized by skin fragility and blistering. The clinical overlap between EB subtypes, coupled with incomplete patient data, complicates classification, prognosis, and management. Genetic analysis can refine diagnoses, particularly in cases with ambiguous phenotypes.

Material and Methods: This study investigated nine Tunisian patients clinically diagnosed with EB, including cases with incomplete data or unclassified subtypes. Exome sequencing (ES) was performed to identify pathogenic variants in EB-associated genes. Bioinformatic analyses assessed variant pathogenicity, facilitating candidate selection for further functional validation.

Results: Eleven pathogenic variants were identified in four genes (COL7A1, LAMA3, DST, and FERMT1), including three novel variants in patients with initially unclassified EB. ES confirmed diagnoses of recessive dystrophic EB and junctional EB in these cases. For patients with incomplete clinical data, genetic findings enabled accurate reclassification, bridging the gap between genotype and phenotype. Bioinformatic predictions validate pathogenicity, reducing the need for extensive functional studies. Integrating ES with bioinformatics significantly streamlined diagnostics, minimizing time and costs compared to traditional serial testing.

Conclusion: ES is an essential tool for EB diagnosis, particularly in cases with incomplete clinical data. Its combination with bioinformatic analyses enhances diagnostic precision, ensuring molecular findings align with clinical phenotypes. This study highlights the importance of integrating genetic approaches into EB diagnostics to improve patient management through early, accurate, and cost-effective classification.

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P16 Diagnostic Improvements and Quality Control

P16.001.D Evaluation and optimisation of the variant prioritisation algorithm for whole genome sequencing-based newborns screening in the Generation Study

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Background: The Generation Study explores the benefits, challenges, and feasibility of using whole genome sequencing to screen 100,000 newborns in England for >200 severe, early onset conditions across > 450 genes in which early, equitably accessible interventions could contribute to treatment success. Additionally, the genomes will be used to support research.

Material and Methods: To maximise positive predictive value and minimise downstream manual review requirements, we have developed an automated variant prioritisation pipeline that prioritises reported pathogenic variants from ClinVar, a commercially procured dataset, specialist-curated variant lists, and predicted loss-of-function variants. Testing on 34,410 individuals of mixed ancestry, not enriched for rare disease, identified the highest numbers of prioritised non-reportable variants in genes associated with multiple phenotypes, multiple modes of inheritance, overlapping other disease genes and/or hypomorphic variants. Specificity was then assessed in a 5,855 genome cohort and 1,362 genome cohort with the optimised pipeline for use in the study.

Results: Specificity was estimated at 95–97% across the cohorts, meaning 3–5% of the genomes will need manual review of prioritised variants, while ~1% of cases are expected to have reportable variants. Study-wide sensitivity, assessed in a 546 patient cohort with known diagnostic variants, reached 80%, primarily missing missense variants absent in known variant datasets. Variant callability was assessed using genome alignment quality and coverage per-gene.

Conclusion: In summary, we demonstrate the value of analytical validity assessment of genes screened in the study, and the potential of our bioinformatics pipeline to prioritise the majority of diagnostic variants whilst minimising false positives.

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P16.002.A Integrative multi-omics for undiagnosed Rare Diseases (Omics-RD)

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Background: Over 100 unsolved rare disease (RD) cases with inconclusive WES/WGS results—including leukodystrophies, spastic paraplegias, inherited metabolic disorders, and other complex syndromes—were included in the OMICS-RD project. The goal was to apply complementary omics technologies to prioritize disease-associated genes and causal variants.

Material and Methods: RNA-seq was performed on 83 blood and 28 fibroblast samples. The DROP pipeline was used to detect aberrant gene expression and splicing. Long-read WGS was conducted on 20 trio families (60 individuals total). Variant calling for SNVs, CNVs, SVs, and STRs was performed using the epi2me wf-human-variation pipeline, while methylation call was carried with modkit based on dorado's basecalling.

Results: Multi-omics analysis helped reveal the effects of causal mutations, including a a) homozygous duplication leading to a twofold increase in gene expression, b) SNV affecting splicing, c) a 40 kb deletion disrupting homologous exons of two consecutive genes, resulting in a gene fusion, d) a 50 kb heterozygous deletion causing a 50% reduction in gene expression, e) a rare intronic variant leading to intron retention and a 50% decrease in expression, f) a transposon insertion within an intron, g) a deep intronic variant inducing exon truncation, h) a triplet expansion causing an 80% decrease in expression, and i) a case with probable maternal UPD.

Conclusion: RNA-seq proved invaluable for detecting the functional consequences of previously deprioritized DNA variants, while long-read technologies enabled haplotype phasing, epivariation detection, and the identification of complex structural variants.

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P16.003.B Feasibility, acceptability and clinical outcomes of genomic newborn screening in a prospective cohort

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Background: Incorporating genomic sequencing into newborn screening (NBS) has the potential to dramatically increase the number of detectable conditions, but raises a range of practical and ethical issues. There is currently limited evidence from prospective cohorts internationally to guide policy and implementation.

Material and Methods: The BabyScreen+ study investigated the feasibility, acceptability and outcomes of clinically accredited genomic NBS (gNBS) in a prospective cohort of 1,000 newborns in Victoria, Australia. Prospective parents enrolled during pregnancy. Newborns were screened for variants in 605 genes associated with early onset, severe, treatable conditions using whole genome sequencing (WGS) on DNA from dried blood spots (DBS). Our semi-automated workflow identified variants for further consideration and reporting. Assay failure rates and causes, turnaround times, and clinical impacts of results were captured. Experiences of participating families were explored using surveys and interviews.

Results: gNBS was performed in 1,000 newborns, of whom 16 (1.6%) were identified as having a high chance of being affected by one of the conditions screened. Of these, only one condition was detectable by standard NBS. Average time to result was 13 days. Challenges with our approach included insufficient DNA yield from DBS (2%), data quality issues (19%), and a high manual review burden (55%). The median decisional regret was low, and >99% of participants believed gNBS should be available to all parents.

Conclusion: We have demonstrated the feasibility of clinically accredited gNBS for over 600 genes, with high accuracy and acceptability. Further work is needed to address issues of scalability and equity.

C.O.I. Disclosure:

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P16.004.C Performance of short-read whole-genome sequencing (srWGS) for detecting short tandem repeat

expansions: a comparison with standard of care

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Background: Short tandem repeat (STR) expansions cause several hereditary disorders, including myotonic dystrophy type 1 (DM1), C9orf72-related frontotemporal dementia and/or amyotrophic lateral sclerosis, fragile X syndrome (FXS), and Huntington disease (HD). Standard of care (SOC) diagnostics commonly rely on PCR-based methods and/or Southern blot, but whole-genome sequencing (WGS) is emerging as a potential alternative for high-throughput screening. This work evaluates the performance of short read WGS (srWGS) compared to SOC for STR expansion detection.

Material and Methods: We analyzed 180 STR loci in 142 patients using both srWGS (Illumina platform, in-house pipeline based on ExpansionHunter) and SOC (e.g., PCR-based methods and/or Southern blot). Loci analyzed included *AR, ATN1, ATXN1, ATXN10, ATXN2, ATXN3, ATXN7, ATXN8OS, C9orf72, CACNA1A, CNBP, DMPK, FMR1, FXN, HTT, NOP56, PABPN1, PPP2R2B*, and *TBP*. Concordance was assessed at two levels: (1) whether srWGS correctly classified expansions in clinically relevant categories (e.g., normal, intermediate, premutation, or full mutation), and (2) whether srWGS correctly distinguished normal repeat lengths from any expansion (e.g., intermediate, premutation, or full mutation).

Results: (1) SrWGS was concordant with SOC in 160/180 (88.9%) analyses for clinical classification of expansions. (2) When distinguishing normal repeat lengths on srWGS from any expansion, concordance improved to 97/97 (100%).

Conclusion: SrWGS demonstrates high concordance with SOC in detecting STR expansions and shows promise as a first-tier screening tool. While refinements are needed for precise classification of expansion categories, srWGS reliably rules out clinically significant expansions in the examined loci when repeat length is classified as normal.

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P16.006.A Harmonized framework for RNA-seq-based rare disease diagnostics in a pan-continental consortium - Solve-RD (Award Candidate)

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Background: To enhance the diagnosis of rare disease patients within the European Solve-RD project, multi-omics data, including DNA, RNA-seq, and clinical phenotype, were integrated. Given the complexity of the data and the involvement of multiple analysts and interpreters from five European Reference Networks, the need to optimize and standardize result presentation became evident. Additionally, a structured framework for data interpretation was essential to ensure consistency.

Material and Methods: We detected aberrant expression and splicing in RNA-seq data from 350 affected individuals who were initially undiagnosed after DNA sequencing. These outliers were integrated with variants, clinical phenotype, and segregation data into a per-family report containing annotated result tables and plots. Close collaboration between bioinformaticians and clinical geneticists was essential and performed through regular consultation hours and "Solvathon" workshops. We developed a new framework to standardize the integration and classification of the results within the Solve-RD network.

Results: A median of 10 expression and splicing outliers were reported per sample. Most of them were discarded, mainly due to phenotype incompatibility. Eighteen families received a diagnosis, corresponding to a 7% diagnostic rate—comparable to the 12.6% achieved globally at Solve-RD through DNA analyses. Additionally, over 80 candidate variants were identified, along with five potential new disease genes.

Conclusion: The additional solved cases highlight the value of RNA-seq in rare disease diagnostics. Our framework efficiently filters and classifies RNA outliers, streamlining the diagnostic process. This approach is particularly beneficial for large consortia, such as the UDN and the emerging ERDERA.

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P16.007.B Genome sequencing for the diagnosis of intellectual disability as a paradigm for rare diseases in the French healthcare setting: the prospective DEFIDIAG study

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Background: Intellectual disability (ID) is the first cause of referral to medical genetic departments in French academic hospitals. Growing evidence of the effectiveness of genome sequencing (GS) compared to reference sequencing strategies (RS) (fragile X expansion testing, chromosomal microarray analysis and 44 ID genes panel) is now well established. However, the value of GS as the first all-in-one test in ID has yet to be confirmed at national level.

Material and Methods: DEFIDIAG is a national prospective pilot study carried out in the framework of the French initiative for genomic medicine which aimed at comparing the diagnostic yield of GS *trio* analysis (GST) with the RS in real-life conditions of clinical and laboratory workflows. Both strategies were applied blindly in 1239 ID probands (50% previously investigated), including 187 patients randomized to undergo GS *solo* in addition to GST and RS.

Results: Overall, 442 class 4/5 SNVs were identified, 171 class 3+ SNVs, 79 class 4/5 CNV and 10 class 4/5 structural variants. The diagnostic yield for class 4/5 variants increased from 17.3% with the RS to 41.9% with GST in the never explored patients, and from 21.9% to 29.9% with GS *solo*. In addition, a prompt data reanalysis led to the identification of a recurrent pathogenic de novo variant in the *RNU4-2* gene in 7 patients.

Conclusion: The DEFIDIAG study demonstrates that GS as the first-line diagnostic test for ID shows a significantly better diagnostic yield, including in *solo* situations, compared to the RS in real-life medical genetic care.

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P16.008.C Does quality of whole genome analysis matter?

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Whole-genome sequencing (WGS) using next-generation platforms is widespread and essential for the 1+Million Genomes Initiative. Laboratories performing professional WGS typically seek ISO17025 or ISO15189 accreditation to ensure regulatory compliance which involves verifying and validating their entire process and demonstrating proficiency. Currently, no proficiency testing schemes for the full WGS process exist. Interlaboratory comparisons (ILC) provide an alternative to demonstrate competence.

We conducted an WGS ILC with 19 European laboratories using Illumina NovaSeq6000 short-read sequencing, evaluating 57 quality metrics for laboratory sequencing performance, sample preparation and bioinformatics, with additional investigative metrics for process improvement. To ensure relevance, we distributed blood-extracted gDNA from a healthy donor - closely mirroring the intended use sample type - and alongside, the well-established GIAB reference gDNA. Gold-standard blood-extracted gDNA variant sets for SNVs, indels, and SVs were established using 600x short-read sequencing, orthogonal nanopore sequencing and variant transmission. Results showed blood-derived gDNA provided more uniform coverage than cell-line DNA. PCR-free sample preparation methods reduced indel false positive and negative calls 10-fold. In both testing items, SV calling exhibited shocking discrepancies in true-positive, false-positive, and false-negative calls across pipelines.

These findings highlight the importance of using relevant testing items and favouring PCR-free methods. SV detection with short reads remains challenging and requires substantial improvement. Proficiency testing is crucial to demonstrate competence, maintaining vigilance, pinpoint frailties and improve aspects of sequencing workflows. This ILC challenges the perception that WGS is a solved problem, demonstrating that major pitfalls persist even with common NGS methodologies.

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P16.009.D Long-read sequencing outperforms Sanger sequencing for RT-PCR based RNA splicing studies in a clinical setting

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Background: Genetic variants altering mRNA splicing contribute up to 9% of variants underlying genetic diseases, which is biased towards canonical splice sites, and likely an underrepresentation. Continued improvements in long-read sequencing technologies promise a sensitive and high-throughput method for identifying multiple RNA transcripts resulting from a single splice altering variant.

Aim: To validate Oxford Nanopore technology (ONT) long-read sequencing as a read-out after RT-PCR for potential splice variants identified in research or diagnostics.

Methods: Targeted RT-PCR was performed on RNA from blood samples of the index patient and at least 2 controls for each variant. RT-PCR cycling conditions were adjusted based on GTEx values in whole blood to reduce PCR bias. These amplicons were sequenced in-house using ONT. Reads were mapped and interrogated using Integrative Genomics Viewer (IGV). Allele specific transcripts were quantitated using informative SNVs and then compared to diagnostic Sanger-determined splicing effects.

Results: Of 102 variants tested by RT-PCR and ONT sequencing, 73 had previously undergone Sanger sequencing. While these approaches were 98.6% concordant in the detection of a splice-effect (only 1 low-abundant mis-splicing event was missed by Sanger), the increased sensitivity of ONT sequencing uncovered additional RNA transcripts in 8 assays. For an additional 7 assays which had failed PCR and/or Sanger sequencing reaction, ONT was successful in generating informative reads for clinical interpretation.

Conclusions: In this ongoing validation study, ONT outperformed the current diagnostic Sanger analysis, simplifying the detection of aberrant transcripts and interpretation of splice-altering variants.

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P16.010.A When timing is essential: application of next-generation sequencing (NGS) technologies to provide a molecular diagnosis for critically-ill children admitted to Neonatal and Paediatric Intensive Care Units (NICUs/PICUs)

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Background: Up to 1/3 of critically-ill infants present a genetic disorder and NGS technologies are the best approach to rapidly diagnose these rare and clinically heterogeneous diseases.

Material and Methods: Three NGS-based pilot studies were conducted in the Italian intensive care setting. At (1) IRCCS "Burlo Garofolo" (Trieste), 26 neonates were enrolled; at (2) ASST "Papa Giovanni XIII" (Bergamo), 237 children were analysed; at (3) IRCCS "Ospedale Pediatrico Bambino Gesù" (Rome), 300 paediatric patients were recruited.

Results: At (1), 62% of cases were solved through Whole Exome Sequencing (WES) analysis. For instance, a heterozygous paternally inherited variant in the *ABCC8* gene was identified together with the somatic loss of the maternal 11p15 region: the molecular diagnosis of focal familiar hyperinsulinism was essential to guide the surgical management. At (2), Whole Genome Sequencing achieved a diagnostic yield of 50%, including the identification of a homozygous deletion in the *LIPA* gene in an infant with acute metabolic decompensation, which allowed to promptly start the specific enzymatic replacement therapy. At (3), WES showed a diagnostic yield of 65%: for example, by highlighting a homozygous variant in the *SCYL1* gene and posing a molecular diagnosis of CALFAN syndrome, we provided precise information on the neurological prognosis.

Conclusion: After these promising results, we will launch the "Baby Wolf" study, the first Italian multicentre project that, through a shared WGS-based protocol, aims to achieve a molecular diagnosis in critically-ill NICU/PICU patients, thus providing tailored management plans and appropriate family counselling in a timely and cost-effective manner.

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P16.011.B Translational Diagnostics and Therapeutics Program (TDTP): six years of experience in clinical functional genomics

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Background: Rare diseases pose significant challenges in identifying and interpreting the pathogenicity of genetic variants,

often leading to delayed or missed diagnoses. In our hospital, the Translational Diagnostics and Therapeutics Program (TDTP) addresses these challenges to validate genetic variants in the diagnostic process.

Material and Methods: The TDTP pipeline for diagnostic decision-making includes phenotype assessment and candidate variant analysis by in-silico studies and experimental functional genomics.

Results: The TDTP has assessed 274 cases from different pediatric specialities of which 211 have been evaluated for diagnostic purposes. The diagnostic yield of the concluded cases (n = 175) was 70.28% (n = 123). The high diagnostic rate can be attributed to our precision phenotyping approach, combined with in-silico studies and/or experimental functional genomics. Patients were selected based on nine specific criteria, the most common being carriers of genetic variants of uncertain significance (n = 75). In-silico analyses were the most frequently studies, involving the examination of DNA, RNA, and protein to predict genetic variants pathogenicity and their impact on the function and 3D structure of the encoded proteins (n = 155). In 76 patients, we performed experimental functional genomics using fibroblasts or peripheral blood samples to reach a diagnosis. The experimental methods included RT-qPCR (n = 23), Sanger sequencing (n = 21), Western blotting (n = 22), and computational imaging analysis of immunostained samples (n = 23). Blood samples were adequate for RNA analyses, offering a more practical approach for routine clinical practice and speeding up result delivery.

Conclusion: The program validates the effectiveness of intramural scientific translational approach in addressing diagnostic challenges in rare diseases.

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P16.012.C Towards routine modopathy diagnostics: advances and clinical application of direct RNA sequencing by Oxford Nanopore Technologies

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Background: RNA modifications play a crucial role in gene regulation and have been implicated in disease mechanisms (modopathies). The *METTL5* gene encodes a methyltransferase responsible for site-specific rRNA methylation. Pathogenic variants in *METTL5* cause aberrant methylation and are associated with intellectual developmental disorder. However, detecting aberrant RNA modifications in the absence of clear pathogenic variants remains challenging in routine diagnostics. Direct RNA sequencing (DRS) by Oxford Nanopore Technologies enables RNA analysis in its native form, offering full-length isoform detection and unique insights into epitranscriptomic features, with potential applications in modopathy diagnostics.

Material and Methods: We utilized DRS with RNA004 chemistry and novel basecalling models for Ψ and m6A to analyze RNA from human cell lines, synthetic oligonucleotides, and blood samples, comparing performance to earlier sequencing chemistries. As a proof of concept, we analyzed blood-derived RNA from a patient with a truncating pathogenic *METTL5* variant and a potential splice VUS to assess both its splicing effect and impact on RNA methylation.

Results: RNA004 chemistry significantly improved throughput and accuracy, enabling precise site-specific RNA modification detection. For the first time, we demonstrated that DRS can validate a VUS by integrating RNA modification analysis. DRS confirmed aberrant splicing and loss of methylation at the *METTL5*-specific rRNA site, supporting the pathogenicity of the variant combination.

Conclusion: This study highlights the potential of DRS for modopathy diagnostics and VUS validation. By combining splicing analysis with RNA modification profiling, we provided the first functional validation of a VUS, paving the way for routine clinical implementation.

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A. Wierczeiko: None. **C. Hewel:** None.

P16.013.D Accurate HBA and SMN genotyping within an integrated NGS workflow: a step toward accessible carrier screening

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Background: The widespread adoption of carrier screening is impeded by high costs and fragmented analytical workflows. Alpha Thalassemia and Spinal Muscular Atrophy (SMA) are among the most prevalent carrier conditions worldwide. Their respective HBA and SMN genes occur in complex genomic regions, necessitating separate analyses, increasing costs and workflow complexity.

Material and Methods: Validation study on targeted callers for SMN and HBA genes implemented in the DRAGEN™ framework along with spike-in panel supplementing the Illumina DNA Prep with Exome 2.5 Enrichment. Positive cell lines for [HBA (α3.7, α4.2, and two copy deletions/duplications)] and SMN1/2 (deletions/duplications) were used to assess repeatability. 71 blinded clinical samples of [7 positive for SMN1 exon7 deletion] and [11 positive for HBA -α3.7,-α4.2, -α20.5] were used to evaluate reproducibility.

Results: Applying the novel solution to a cell line cohort with orthogonally confirmed results for HBA (710 datasets from 203 unique samples) and SMN (728 datasets from 216 unique samples), demonstrated 100% (710/710) concordance for HBA and 97.9% (713/728) concordance for SMN1/2. Discordant results relate to cell line artifacts. In a separate clinical sample cohort (182 datasets from 71 unique samples), the concordance with matched MLPA was 100% (182/182) for HBA and 100% for SMN1 (182/182).

Conclusion: The novel callers and spike-in panel demonstrate a reliable approach for profiling HBA and SMN genes within an integrated NGS workflow. This method has the potential to reduce reliance on orthogonal assays in carrier screening, enhancing cost-effectiveness and accessibility.

For Research Use Only. Not for use in diagnostic procedures.

C.O.I. Disclosure:

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P16.014.A Integration sites and functional implications of novel processed pseudogenes in mendelian diseases

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Background: Processed pseudogenes are genomic elements that arise through the retrotransposition of mRNA molecules. Due to their origin, they lack introns and often contain poly-A tails, reflecting their formation from reverse-transcribed RNA. While CNV caller tools can detect them as multiple intron deletions of their parental genes, their precise genomic integration sites could easily be overlooked in bioinformatic analyses.

Material and Methods: We analyzed 3,655 whole-genome datasets from probands with suspected Mendelian diseases and their healthy relatives. The data were processed using bioinformatic pipelines to identify and annotate processed pseudogenes, with a particular focus on their integration sites and potential functional implications.

Results: We detected 34 processed pseudogenes that are absent in the GRCh38 genome reference, 25 of which are novel and have not been described previously. For 22 of these pseudogenes, we were able to determine the precise Insertion site, while 3 were reclassified as potential mapping errors. Most of the revealed processed pseudogenes have low allele frequency (29 events with a frequency below 0.5%). Notably, we observed that 7 pseudogene integration events occurred within intronic regions, which could lead to potential regulatory or splicing abnormalities in host genes.

Conclusion: Processed pseudogenes are relatively rare genomic events; however, they can contribute to monogenic diseases or serve as valuable markers for population genetics due to their low frequency and unique integration patterns. Further investigation into their functional impact and distribution across populations will enhance our understanding of their role in human health and evolution.

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P16.015.B Implementing RNAseq analysis alongside WGS in healthcare, a diagnostic workflow for patients with rare diseases

Lucía Peña-Pérez[1], *Anders Jemt*[2], *Jesper Eisfeldt*[3], *Henrik Stranneheim*[2], *Nicole Lesko*[3], *Daniel Nilsson*[1], *Chiara Rasi*[2], *Anna Wedell*[1], *Valtteri Wirta*[2], *Anna Lindstrand*[1], *Håkan Thonberg*[1]

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Background: RNA-seq has emerged as a powerful complementary technique to whole genome sequencing (WGS) for rare disease diagnostics. While WGS provides extensive genomic information, it often fails to elucidate functional consequences of genetic variants, leaving patients undiagnosed. Performing RNA-seq in combination with WGS enables direct assessment of variant effects on transcripts, potentially increasing the diagnostic yield. Here we report the full implementation of RNA-seq for blood transcriptome analysis. We present the entire workflow from blood-sampling to visualization combined with WGS for interpretation, including a pilot clinical test of 20 patient samples referred for diagnostic analysis.

Material and Methods: Blood samples from 215 rare disease patients were collected in PAXgene tubes and RNA was isolated, sequenced, and processed using our in-house-developed bioinformatic pipeline Tomte. A total of 195 samples were used to create a database for comparing gene expression and splicing events between controls and patients. Tomte performed allele-specific variant calling and annotation, and detected allelic imbalance, aberrant expression, and aberrant splicing. The results were then integrated with previously obtained WGS data and displayed in Scout, our in-house-developed decision support tool for variant interpretation.

Results: Our pilot study of 20 samples revealed an average of five aberrant splicing and eight aberrant expression events per patient. This included cases of reduced exon usage and haploinsufficiency linked to previously identified WGS variants.

Conclusion: The integration of RNA-seq with WGS has the potential to increase the diagnostic yield for rare disease patients by directly showing the functional impact of variants on transcripts.

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P16.017.D Diagnostic utility of optical genome mapping in constitutional cases - a single institutional experience

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Background: Current standard-of-care (SOC) methods to detect structural and copy number variants in constitutional disorders include G-banding, fluorescence in situ hybridization (FISH) and chromosomal microarray. The limitations of SOC methods often require multiple tests to determine the underlying chromosomal rearrangement(s). Optical genome mapping (OGM) can overcome these limitations by being able to detect balanced structural rearrangements, copy number variants, and unbalanced structural variants at a higher resolution in a single assay. Our goal was to evaluate the diagnostic utility of OGM through comparison with traditional cytogenetic methods using samples representing a variety of chromosomal rearrangements.

Material and Methods: OGM was performed using the Saphyr system (Bionano Genomics) on 29 pediatric patients and compared to previously identified variants detected by Cytoscan HD SNP Array (Affymetrix), karyotype and/or FISH results.

Results: Comparison of OGM and previous cytogenetic findings revealed concordance in 25 samples from the validation cohort. Four samples were not included in the validation set, as they had insufficient DNA or failed quality metrics. In total, OGM identified 67 variants, including previously identified inversions, deletions, duplications, dicentrics, and homozygous gains and losses. OGM was able to resolve suspected balanced translocations, identify

inversions, and additional variants that were previously undetected by other methods.

Conclusion: OGM provides a comprehensive higher resolution platform for detecting clinically relevant chromosomal rearrangements. OGM also identified additional variants, suggesting increased sensitivity will result in a higher diagnostic utility for constitutional disorders. Parameters are currently being optimized for integration of OGM into the clinical workflow.

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P16.018.A Non-invasive prenatal testing for monogenic disorders using whole-exome sequencing of cell-free DNA

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Background: Non-invasive prenatal testing (NIPT) has transformed prenatal screening by analyzing cell-free DNA (cfDNA) from maternal plasma to detect common fetal chromosomal aneuploidies. However, many severe postnatal genetic disorders result from dominant de novo pathogenic variants in single genes, which are not detected by standard NIPT procedures. Advances in next-generation sequencing and targeted gene panels have shown technical feasibility of identifying such pathogenic variants with high accuracy. Despite that, NIPT for monogenic disorders has not yet become part of routine clinical practice in Czechia. Expanding NIPT to include monogenic conditions holds a promise of improving early diagnosis and pregnancy management.

Material and Methods: cfDNA was extracted from maternal plasma using the QIAamp Circulating Nucleic Acid Kit. Sequencing libraries were prepared using the Twist cfDNA Library and UMI Adapter System, followed by enrichment with a Twist Exome panel. Sequencing was performed on the Element Biosciences AVITI system using a High Output kit.

Results: A complete workflow for monogenic NIPT analyses was established. Whole-exome sequencing (WES) successfully confirmed dominant pathogenic variants in disease-related genes in cfDNA samples. Estimation of fetal fraction was performed using allele frequency analysis.

Conclusion: Our proof-of-concept study highlights the feasibility and potential of cfDNA-based WES for NIPT of autosomal dominant monogenic disorders, such as achondroplasia. Integrating NIPT of monogenic disorders into routine care could greatly improve early diagnosis, optimize allocation of healthcare resources, and support informed parental decisions. On the other hand, future studies need to focus on the NIPT-related ethical issues as well.

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P16.019.B Improvement of management and turnaround time of NGS based genetic testing by using an all in one digital solution

***S. Gambardella**[1,2], G. Macari[3], L. Cafforio[4], R. Ferese[1], R. Campopiano[1], M. Chiaravalloti[1], E. Agolini[5], M. Mattiuzzo[6], M. Lodi[7], M. Dentici[7], S. Gardini[4], B. Dallapiccola[7], A. Novelli[8];*

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Background: NextGenerationSequencing diagnostic workflows are complex and various challenges related to patient and sample management must be addressed to enhance clinical assessments, laboratory processes and accelerate diagnoses.

Methods: We conducted a pilot study to evaluate the time efficiency of a diagnostic process using an end-to-end digital solution, compared to the traditional workflow. The study involved 80 patients with rare diseases recruited at Bambino Gesù Children's Hospital-Rome and Neuromed Institute-Pozzilli. We performed a swimlane analysis of the following steps: genetic counseling and request submission by clinical geneticists(1), sample check-in and analytical processing by laboratory technicians(2) and variant analysis,interpretation and reporting by biologists(3).

Results: Our results showed that the average turnaround time for the traditional workflow was significantly longer (14d) compared to the digital solution-based workflow (5d). The digital solution streamlined the diagnostic process by incorporating an all-in-one system that facilitated digital genetic counseling, easy submission of genetic requests, digital laboratory operations, sample tracking, NGS data analysis, variant prioritization, automatic generation, validation and delivery of reports.This integration led to an overall 65% reduction in TAT: 15% in phase1, 20% in phase2, and 30% in phase3.

Conclusions: We found that the end-to-end digital solution improved laboratory efficiency and productivity. This approach created a more reliable and scalable laboratory, enhanced patient and sample management, streamlined information sharing, reduced errors, and sped up times. Furthermore, the continuous workflow enabled by the digital solution contributes to the growth of a clinical and biological dataset, facilitating new gene-phenotype association studies, correlation research and enhanced diagnosis.

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P16.020.C The Gaslini Genomic Neonatal Screening for the early diagnosis of rare diseases

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Background: Newborn screening (NBS) is a program aimed at detecting pre-symptomatic newborns with serious yet treatable

disorders. Advances in molecular genomics and gene therapy are expected to expand the spectrum of conditions included in NBS. We evaluated the impact of whole genome sequencing (WGS) in the NBS program for the early identification of infants with potentially treatable diseases or carriers of recessive diseases.

Material and Methods: We performed Illumina PCR-free WGS on DNA extracted from dried blood spots of 200 healthy newborns collected at the Gaslini Institute. Detection of SNVs, indels, CNVs, SVs, and expansions, was performed using Dragen Germline, as well as detection of copy number of SMN1 and SMN2. Variants were annotated and filtered using the in-house BioGeAR pipeline (https://github.com/igg-bioinfo). Analysis focused on ClinVar pathogenic variants within 177 genes associated with disorders potentially included in Italian NBS (e.g., SMA, SCID, CF, Pompe disease). Analyses were automated to reduce turnaround time.

Results: WGS pipeline was validated on 24 individuals with known pathogenic variants. WGS of the first 190 newborns enrolled in the study, with an acceptance rate of 99%, identified homozygous and compound heterozygous clinical Pathogenic/Likely Pathogenic variants in recessive genes (SMN1, CFTR, BTD) in 3 newborns. Gene therapy was administered to the patient with SMA disease.

Conclusion: Our findings indicate that genomic NBS enables the early detection of genetic factors underlying metabolic dysregulation and other disorders, potentially influencing clinical management in the neonatal period. We also identified ethical issues that should be addressed before wider implementation of genomic NBS.

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P16.021.D Impact of Gene Specific Guidelines on the Interpretation of Variants of Uncertain Significance

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Introduction: The ClinGen consortium has recently published various gene-specific and disorder-specific variant interpretation criteria, refining the original ACMG guidelines. This study evaluates the impact of applying these criteria on the classification of variants of uncertain significance (VUS).

Methods: Guidelines were extracted from the ClinGen Criteria Specification registry (CSpec) and analyzed for scoring modifications compared to the standard ACMG framework. Variants initially classified as VUS under ACMG guidelines were reanalyzed using the gene-specific criteria to assess changes in classification.

Results: A total of 106 CSpec guidelines were identified. Overall these guidelines applied stricter frequency cutoffs, stringent thresholds for prediction tools, and refined hotspot definitions, generally reducing pathogenic strength. Among 332 VUS analyzed across 14 genes, 196/332 (59%) demonstrated a reduction in pathogenic strength, leading to a downgrade of 68/196 (35%) VUS variants to likely benign (LB) classification. Only 22 VUS (7%) showed increased pathogenicity probability, but none reached a likely pathogenic (LP) classification. For 114/332 (34%) variants, the pathogenicity probability remained unchanged. BRCA1/BRCA2 showed the highest reclassification rate, with 19/20 VUS variants downgraded to LB, while the impact was minimal for PMS2, affecting scores of 15/32 variants, with 3 variants with classification alterations.

Conclusions: Applying CSpec guidelines significantly reduced pathogenicity scores, reclassifying 18% of VUS as LB. This demonstrates the utility of specific guidelines in reducing uncertainty while requires the need for more advanced analytical pipelines for comprehensive assessment.

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P16.022.A Enhanced precision in predicting the RNU4-2-related phenotype (ReNU syndrome) by facial image analysis of multiple family members

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Background: The introduction of trio genome sequencing (TGS) has revolutionized the identification of de novo mutations in sporadic cases of intellectual disability. In particular, the discovery of non-coding variants underlying the recently delineated ReNU phenotype would not have been possible without TGS. However, as TGS is not widely accessible, there is an increasing demand for scalable and cost-effective screening methods. We therefore fine-tuned GestaltMatcher for this purpose. GestaltMatcher is an artificial intelligence that analyzes phenotypic features of patients with rare diseases.

Material and Methods: GestaltMatcher was trained on over 12k facial photographs including portrait images of 35 unrelated individuals with molecularly confirmed ReNU syndrome. In addition, we analyzed the facial encodings of unaffected family members within this clinical face phenotype space and subtracted their feature embeddings from the patient data. This preprocessing step effectively removes family-specific but non-pathogenic facial traits.

Results: We assessed the rank of the correct diagnosis in GestaltMatcher before and after integrating the embeddings from unaffected family members. We find that including imaging data of additional relatives, ideally taken at a similar age, can be used to increase the signal-to-noise ratio. In average, the rank of ReNU increased from rank 10 to rank 2.

Conclusion: Portrait photos of unaffected family members significantly enhance the accuracy of ReNU syndrome prediction, making computer assisted image analysis a promising screening approach for tiered diagnostics. Through this increased sensitivity towards ReNU, targeted Sanger sequencing of the RNU4-2 locus may serve as a rapid and cost-effective alternative to whole-genome sequencing.

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P16.023.B Reasoning with AI: simultaneous HPO extraction and disorder classification for syndromic faces

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Background: Next-generation phenotyping approaches have demonstrated high accuracy in suggesting diagnoses based on dysmorphic facial features. We propose a novel approach using convolutional neural networks (CNNs) to simultaneously infer Human Phenotype Ontology (HPO) terms and classify disorders based on syndromic faces. By integrating HPO extraction and disorder prediction, our method enables cross-validation of results, improving diagnostic robustness. We aim to enhance both diagnostic support and explainability in AI-driven rare disease classification.

Material and Methods: Through GestaltMatcher Database, we set up a large-p effort to annotate the presence and absence of HPO-terms. As annotation of HPO-terms is a subjective process, we allowed multiple experts to annotate the same images to improve the overall robustness. By simultaneously learning to predict disorder and infer HPO-terms, we can cross-reference inferred terms with disorders to (in)validate predictions. Further, since HPO-terms inherently carry spatial information, we can gain new insights into the predicted disorders.

Results: 1000 images, across 10 disorders were annotated with 72 common HPO-terms for the disorders, alongside any term the annotators considered important. We trained several CNNs and compared the performance when predicting HPOs, disorders, and a combination of the two. The latter allowed us to combine the predictions to further improve performance and explainability.

Conclusion: Our proposed approach allows for more and better analyses to be conducted straightforwardly. Further, it inherently improves the explainability of our AI models, improving their usefulness in clinical settings. Additionally, it reduces the subjectiveness of labeling HPO-terms manually and allows clinicians to work more efficiently.

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P16.024.C Identification of prognostic miRNA biomarkers from neonatal CSF extracellular vesicles in post-hemorrhagic ventricular dilatation via machine learning approaches

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Background: Neonatal post-hemorrhagic ventricular dilatation (PHVD), a serious complication of intraventricular hemorrhage (IVH), is linked to significant neurodevelopmental impairments. Early biomarkers are critical for timely prognosis and intervention. As cerebrospinal fluid (CSF) is routinely drained at the onset of PHVD to reduce the intercranial pressure, we aimed toexplore this understudied, yet accessible biofluid for extracellular vesicles (EVs) isolation and identification of miRNA biomarkers.

Material and Methods: We isolated EVs from 190 CSF samples from 43 neonates diagnosed with PHVD. High-throughput sequencing was used to profile EV-associated miRNAs, generating a novel dataset of miRNA signatures. Machine learning (ML) and artificial intelligence (AI) algorithms were applied to analyze these miRNA profiles and predict neurodevelopmental outcomes at 2 and 3 years of age.

Results: We were able to detect 248 miRNAs consistently in 80% of the samples. Our ML models identified key miRNAs with strong predictive power for cognitive and motor outcomes. These miRNAs showed potential as early, minimally invasive biomarkers for neurodevelopmental prognosis. The identified biomarkers provided insights into the pathophysiology of PHVD and may inform the development of targeted therapies.

Conclusion: This study highlights the potential of EV-associated miRNAs as predictive biomarkers for neurodevelopmental outcomes in neonates with PHVD. Further validation in larger cohorts could establish these miRNAs as valuable clinical tools for early diagnosis and prognosis, offering new avenues for mitigating long-term neurodevelopmental deficits.

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P16.026.A Efficacy of universal genome sequencing in critically ill infants on extracorporeal membrane oxygenation

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Background: Rapid whole genome sequencing (WGS) in neonatal intensive care units (NICUs) provides fast, actionable diagnostics but lacks clear timing guidelines, especially for infants on extracorporeal membrane oxygenation (ECMO). With growing evidence that early genetic insights could improve outcomes in complex ECMO cases, this study assesses the diagnostic efficacy of universal WGS in this high-risk group.

Material and Methods: We conducted a prospective, multicenter, non-interventional pilot study across eight ICUs within the Children's Hospitals Neonatal Consortium (CHNC) using a single IRB reliance model. The study targeted enrollment of 25 infants (0–12 months) with the primary selection criterion being the need for ECMO. Blood samples were collected prior to cannulation with parental consent. Genome sequencing and variant analysis followed standard research protocols, with no provisions for the timely return of results to clinicians.

Results: The diagnostic rate was 24% (6/25), revealing diverse causal variants including compound heterozygous (1/6), autosomal dominant (1/6), X-linked (1/6), and chromosomal abnormalities (3/6). One positive case carried a second variant of uncertain significance, and another had an ACMG secondary finding. Overall, secondary/incidental findings were reported in 8% (2/25) of infants. Five cases showed potentially pathogenic variants that could not be conclusively assessed due to study setup limitations.

Conclusion: These findings support the potential clinical benefit of universal, first-tier genome sequencing in pediatric ECMO cases. A future multicenter randomized trial is planned to further evaluate the utility of universal ultra-rapid genome sequencing in this population.

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P16.027.B Common causes of genotyping error; Trends over time

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EMQN, Manchester, United Kingdom.

Background: Genotyping errors have potential to cause patient harm. EMQN asks laboratories to complete a Root Cause Analysis (RCA) for errors identified during External Quality Assessment (EQA). Here, we show trends for the most prevalent causes of genotyping errors.

Method: RCA forms received between 2019–2023 from 358 laboratories were analysed to categorise the root cause of genotyping errors and identify trends over time. Results were sub-grouped by field (germline or molecular pathology testing), and error type (false positive or false negative results).

Results: Over 99% of laboratories that submitted forms following a genotyping error successfully identified root causes. Overall, the leading cause of genotyping error was transcription errors, followed by sample swaps and commercial kit performance.

Trends over the five-year period demonstrated consistent decline in errors resulting from suboptimal bioinformatics pipelines and variant misclassification. However, errors associated with NGS quality control (QC), including filtering parameters and insufficient coverage, have increased, highlighting evolving challenges in the field.

Incorrect or incomplete methodological approaches, including use of outdated gene panels, were the main cause of error for laboratories performing germline testing. This comprised entirely of false negative results. Higher rates of sample swaps and commercial kit use errors were associated with molecular pathology testing.

Conclusion: EQA can help identify errors proactively so the root cause can be identified, and services improved. This analysis highlights the importance of caution and checks regarding validation of analytical and bioinformatic QC, report writing and sample processing to ensure the accuracy of test results and prevent patient harm.

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P16.028.C Project LANCELOT: A Molecular Newborn Screening of Treatable Conditions

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Background: We conceived the Molecular Newborn Screening of Treatable Conditions (LANCELOT) project for the early identification of well-characterised treatable genetic disorders. Our aim is to develop a customised Next Generation Sequencing (NGS) panel-based Newborn Screening (NBS) focused on genomic regions in which druggable disease-causing mutations are located.

Material and Methods: We interrogated the ClinicalTrials.gov database to identify the genes associated with treatable conditions suitable to undergo our NGS-NBS. The 35 selected genes were included in our panel design provided by Agilent SureDesign platform. To assess the project methodology and validate our panel, we conducted a pilot experiment with 60 anonymous newborns. DNA samples were obtained from dried blood spot and enriched with the Agilent SureSelectXT HS Protocol. The libraries were run on the Illumina NextSeq500. Finally, the raw NGS data were analysed using an in-house bioinformatic pipeline.

Results: The data analysis showed a target regions average coverage of 1000X, with an even higher depth for the mitochondrial locus, leading to the identification not only of single nucleotide variants but also copy number variants and mosaicism. This results in a well-characterised, manageable, and cost-effective NGS panel which enables the detection of multiple variant types in a single test.

Conclusion: Performed test proved LANCELOT efficacy, achieving a pivotal milestone towards clinical practice. Following the current evidence-based model for NBS and undergoing continual literature updates, our proposal provides with important technological innovation of high clinical utility and significant economic impact, implementing the diagnostic and therapeutic management of paediatric conditions.

C.O.I. Disclosure:

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P16.029.D Challenging the use of proxies in genotyping analysis for *DPYD* and *UGT1A1*

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Background: Dihydropyrimidine dehydrogenase (DPD) and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) are metabolic enzymes on whose genes several variants were identified. Both enzymes are involved in the metabolism of common chemotherapeutic drugs namely 5-fluorouracil (5-FU) reduced by DPD and irinotecan active metabolite (SN38) glucuronidated by UGT1A1. Genetic variations decreasing the activity of DPD and expression of UGT1A1 pose an elevated risk for potentially life-threatening fluorouracil/irinotecan toxicity. Preemptive genotyping is therefore considered critical for the correct dosing and tag SNVs are still used as proxies to define the genotype of causative SNVs considered in 100% LD. For *DPYD* c.483+18G>A and c.1236G>A/HapB3 are proxy for c.1129-5923C>G and for *UGT1A1* *80 is proxy for *28.

Material and methods: Genotyping was conducted using OpenArray panels interrogating *DPYD* c.483+18G>A, c.1236G>A, c.1129-5923C>G and *UGT1A1* c.-364C>T (*80). *UGT1A1* c.-54_-39A(TA)6TAA>A(TA)7TAA (*28) was evaluated via targeted PCR amplification and Sanger sequencing. The correlation between the genotype of the causative and the proxy SNVs was studied in 3189 and 171 samples for *DPYD* and *UGT1A1*, respectively.

Results: Concerning *DPYD*, lack of correlation was detected in 11 samples (0,35%) between c.483+18G>A and c.1129 5923C>G, and 1 sample between c.1236G>A and c.1129-5923C>G. The correlation analyses between *80 and *28 on *UGT1A1* identified 1 non matching sample (0,58%).

Conclusions: The use of proxy SNVs has evident limitations even for variants considered in 100% LD and could lead to incorrect genotyping results. Direct genotyping of causative SNVs should be preferred as best molecular diagnostic practice especially for variants clearly associated with severe and life-threatening side effects.

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P16.030.A Biallelic loss-of-function variants in *ZNF142* are associated with a robust DNA methylation signature affecting a limited number of genomic loci

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Background: Biallelic loss-of-function (LoF) variants in *ZNF142* underlie a clinically variable neurodevelopmental disorder. *ZNF142* is a zinc-finger transcription factor with potential roles in chromatin organization, implying a possible association of ZNF142 LoF with perturbed genome-wide DNA methylation (DNAm) pattern.

Material and Methods: EPIC array-based DNAm profiling of 27 individuals with biallelic *ZNF142* variants, together with 6 heterozygous carriers and 40 controls was performed. Additional 440 controls were used for discovery and validation analyses. A DNAm signature discovery pipeline was applied, and a machine-learning model was trained to classify individuals carrying *ZNF142* variants of unknown significance (VUS).

Results: We identify a specific DNAm signature for biallelic LoF *ZNF142* variants that was successfully used for VUS reclassification. Analyses directed to explore the genome-wide DNAm landscape in patients revealed a reproducible pattern of large DNAm changes at regulatory regions in a small number of genes. The DNAm signature derived from peripheral blood properly classified individuals carrying biallelic LoF *ZNF142* variants when applied to fibroblasts.

Conclusion: Biallelic LoF *ZNF142* variants result in a specific and robust DNAm signature. The identified DNAm pattern suggests occurrence of a methylation disturbance involving a small number of loci that is shared by multiple cell lineages.

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P16.031.B The diagnostic accuracy of FMR1-STR calling from short-read genome sequencing

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Background: Short-tandem-repeat (STR) expansion is involved in several genetic diseases, including *FMR1*-related disorders with an incidence of 14:100,000, and a female carrier frequency of 1:175. While standard *FMR1* testing is performed using targeted PCR-based assays, *FMR1*-screening from short-read genome sequencing (sr-GS) has been suggested as first-tier tool using e.g. ExpansionHunter but its diagnostic validity has not been fully determined. We therefore analyzed diagnostic accuracy of *FMR1*-STR calling.

Material and Methods: We recruited 130 women seeking preconception carrier screening. After PCR-free Illumina GS, data were aligned using DRAGEN (Illumina), including ExpansionHunter for STR calling. In parallel, the CGG-repeat region in exon 1 of *FMR1* was tested using the Asuragen-Amplidex assay.

Results: With a tolerance of +/−2 repeats, ExpansionHunter and PCR results matched in 78% of samples. The ExpansionHunter does not differentiate between a homozygous allele and an allele drop-out. In 25 cases (19%), the detection of only one allele was based on an allele drop-out, which in one case also included a premutation allele. In ~22% of samples, one or both alleles showed a discrepancy >2 (mean 1.6). There was no clear correlation to the average genome coverage. Overall, there were 2 true positives and 1 false negative, resulting in a 67%-sensitivity [95% CI: 9.43–99.16%] and 100%-specificity [95% CI: 97.14–100.00%].

Conclusion: Due to its limited sensitivity short-read GS data are not suitable to exclude *FMR1*-repeat expansions as a stand-alone test. Nevertheless, if a repeat expansion is suspected from GS as a secondary finding, it has a high positive predictive value.

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P16.032.C External Quality Assessment for Hereditary Amyloidosis: Assuring Test Standards to Support the Identification of Patients Eligible for Treatment

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Background: Hereditary amyloidosis is a group of rare conditions characterised by amyloid accumulation impairing organ function. Hereditary transthyretin amyloidosis (ATTRv) - the most common type - is caused by >130 pathogenic variants in *TTR* gene and presents with neurological and/or cardiac manifestations. Several therapies for ATTRv are now available, making proficient diagnosis crucial for improved patient outcomes. We have established a global external quality assessment (EQA) scheme for *TTR* testing to assure diagnostic service quality.

Methods: Thirty laboratories from 21 countries were selected to participate in the pilot EQA. Three DNA samples with corresponding mock clinical referrals were supplied for *TTR* gene analysis. Clinical reports were assessed for genotyping, interpretation, and clerical accuracy by expert assessors.

Results: Twenty-eight laboratories returned clinical reports. All laboratories reported the correct genotype, of these 93% received full marks for genotyping; 87% received full marks for interpretation and 85% received full marks for clerical accuracy. Of note, many laboratories failed to interpret the genotype in the context of the clinical referral.

Conclusion: This pilot EQA scheme has established that the standard of *TTR* testing for hereditary amyloidosis is good. However, areas of improvement, particularly around the accuracy of interpretation of the genotype result, were identified. To date, there are no standardised criteria for genetic analysis and reporting of *TTR* variants in hereditary amyloidosis. This lack of guidance might compromise patient care. We anticipate continued provision of this EQA scheme will improve testing and reporting standards, whilst simultaneously facilitating the development of best practice guidelines.

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P16.033.D Improving rare metabolic disease diagnostics: The impact of enzyme assays and RNA-sequencing as primary multi-omics screening tools

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Background: Conventional genetic diagnostic protocols rely on DNA sequencing as the primary screening method. An alternative strategy - utilizing non-DNA omics as the primary screening tool, followed by DNA sequencing of candidate genes or regions - has remained largely unexplored.

Material and Methods: We re-analyzed a case that had remained negative by conventional 'gene panel sequencing as primary step' approach. We considered the patient's phenotype to define candidate genes, tested the activity of the encoded enzymes and analyzed corresponding transcripts in blood-derived RNA sequencing. We finally Sanger-sequenced a suggestive region and used in silico prediction to interpret the identified variant. All wet lab analyses were done from dried blood spot filter cards.

Results: The clinical information suggested mucopolysaccharidosis (MPS) or a related metabolic disorder. Enzyme activities were normal for *ARSB*, *GUSB*, *IDUA*, *GALNS*, *GLB1*, *MAN2B1*, but below the level of detection for *IDS*. The transcripts for most candidate genes showed no abnormalities, but insertion of an intron 6-derived stretch of 46 nucleotides was observed in the *IDS* transcript. DNA sequencing of this intronic region revealed a hemizygous *IDS* variant (c.880-1180A>T; NM_000202.5), which is predicted to introduce a strong splice donor, ultimately creating a pseudo exon. The variant was classified as likely pathogenic based on ACMG/AMP/ClinGen guidelines.

Conclusion: Our study demonstrates the potential that combining non-DNA primary screening approaches with dried blood spot analysis has for cost-effectively diagnosing rare genetic metabolic disorders. To establish this approach in routine clinical settings, further research involving automated protocols and large-scale validation studies is necessary.

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P16.034.A A critical review of the factor 8 intron 22 inversion assay in the setting of prenatal diagnostics

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Background: Accuracy and precision in diagnosis and reporting are the cornerstone of Genetic Pathology, particularly in the setting of prenatal diagnostics. Haemophilia A is an X-linked disorder resulting from Factor 8 (F8) deficiency. Requests looking to interrogate the 50% chance of a male fetus inheriting a pathogenic F8 variant from their heterozygous mother, is therefore a common prenatal request.

Material and Methods: F8 intron 22 inversions, responsible for severe haemophilia A, were identified by long-range PCR followed by nested PCR. Exclusion of maternal cell contamination (MCC), critical in prenatal testing, was performed by short tandem repeat fragment analysis. Recently, this diagnostic method received critical review following release of a false-positive prenatal result.

The male fetus was born without evidence of F8 deficiency; thus, no clinical harm came from this report, however demanded investigation of the testing method.

Results: A two-pronged contribution was identified. Firstly, the pathogenic F8 intron 22 inversion was falsely represented in the fetus due to the high sensitivity of the nested PCR assay in detecting minute traces of MCC. In addition, PCR primers for the wildtype allele were found to overlap a single nucleotide polymorphism in the general population. The wildtype allele, therefore, was not detected due to allelic drop-out.

Conclusion: The nested PCR method used to detect the F8 pathogenic intron 22 inversion is highly sensitive to MCC, and therefore not suitable in the prenatal setting. This case provides an example of how a false-positive result can spur critical review and diagnostic improvement.

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P16.035.B Haplotype phasing of biallelic*WNT10B*variants using long-read sequencing in split-hand/foot malformation syndrome

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Background: Whole exome sequencing (WES) is the standard technology in clinical genetics for detecting protein-coding variants. Short-read technologies, while highly accurate, cannot resolve repetitive and GC-rich regions, but long-read sequencing can overcome this problem by resolving difficult sequences and providing epigenetic information. Split-hand/foot malformation syndrome (SHFM) is a congenital limb malformation, clinically and genetically heterogeneous. Variants in *WNT10B* are known to cause an autosomal recessive form of SHFM.

Material and Methods: High molecular weight DNA was freshly extracted from the patient's fibroblasts, and long-read PacBio HiFi sequencing results were visualized in the Geneyx software after bioinformatic analysis. Sanger sequencing confirmed the variants and in silico predictions characterized them as pathogenic.

Results: We report a patient born to unrelated parents who was found to be a compound heterozygote for missense variants in *WNT10B*: c.994C>T, p.(Arg332Trp) and c.638T>G, p.(Phe213Cys). The long-read technology enabled us to phase the reads and show the biallelic nature of the variants, supporting the recessive disease model. The maternally inherited variant p.(Arg332Trp) has been previously reported, whereas the paternally inherited variant p.(Phe213Cys) is novel and not present in gnomAD.

Conclusion: Our findings highlight the utility of long-read haplotype phasing, which provides valuable insights in determining the biallelic nature of variants. This can be particularly informative in recessive disorders when parental DNA is not available, when a variant is de novo, or displays mosaicism. As sequencing costs decrease, high fidelity long-read sequencing might become the preferred technology in clinical genetics, serving as a single diagnostically comprehensive genetic test.

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P16.036.C Benchmarking the benchmark: manual revision and high-resolution repeat-structure aware characterisation of 69 clinically relevant tandem-repeat motifs in the GIAB HG002 reference material

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Background: Tandem repeats (TRs) contribute significantly to human genome variability and have clinical importance. Exact characterisation of these loci is of high importance. Benchmarking TR reference material was, therefore, published recently for the GIAB HG002 individual sample, and is available as mapped sequencing reads and corresponding truth variant set. We performed manual curation of the most common clinically relevant motifs on this reference dataset.

Material and Methods: We downloaded the HG002 TR benchmark v1.0 data (BAM and the VCF). On the BAM files we performed TR genotyping of 69 clinically relevant motifs, by the use of two genotyping tools, Dante and Expansion Hunter. For repeat-motif delineation we used the Dante Clinical TR Database (includes exact HGVS description of the relevant TR motifs referenced for the GRCh38). The results were then cross-compared to the downloaded GIAB-VCF reference, both automatically (using Truvari) as-well-as manually, through the IGV and the alignment visualisation interface of Dante.

Results: We found that validation of genotyping is viable for simple TR motifs. Complex TR motifs, however, represent specific problems which are reflected also in the used conventional VCF files and complicate the automatic comparison of datasets.

Conclusion: To complement the VCF description of the variants, we created an easily readable and interpretable description of the HG002 reference genotypes. This includes not only repeat numbers, but also high-resolution repeat-structure characterisation with HGVS nomenclature and visual depiction of each allele. This material may represent an additional benchmarking dataset for laboratories performing TR genotyping.

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P16.037.D Enhancing diagnostic interpretability of GestaltMatcher: Translating facial phenotype distances into probabilities

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Background: Genetic disorders often present with characteristic facial features. Next-generation phenotyping tools can enhance the diagnostic process by leveraging these phenotypic patterns. GestaltMatcher maps facial images into a high-dimensional clinical face-phenotype space (CFPS), quantifying patient similarities via spatial distances. Prior works demonstrate this clustering approach achieves high top-k syndrome ranking accuracy. However, CFPS distances lack intuitive interpretability. We propose

translating these distances into probabilities, representing the likelihood of a syndrome given a patient's facial phenotype.

Material and Methods: For each syndrome *A* in the GMDB, the distance of each patient to the nearest neighbor with syndrome *A* is computed. A logistic regression model, trained on labeled data, transforms these distances into probabilities for new cases. This framework also provides confidence intervals, estimating uncertainty for each probability.

Results: The derived probabilities are interpretable and align well with expert assessments of facial gestalt distinctiveness. For dual diagnoses, the method assigns high probabilities to multiple syndromes, often yielding cumulative probabilities exceeding 1, as opposed to an overall average below 1. Importantly, converting the scores into probabilities has minimal impact on syndrome ranking, preserving GestaltMatcher's diagnostic accuracy. Confidence intervals offer valuable insights into prediction uncertainty, particularly for rare syndromes.

Conclusion: Translating CFPS distances into probabilities enhances the interpretability and clinical utility of GestaltMatcher's output while maintaining its diagnostic accuracy.

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P16.038.A Whole genome sequencing increases the diagnostic yield of neurodegenerative diseases

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Background: Although WGS is increasingly used in diagnostic contexts, the diagnostic yield often does not exceed 25–30%. This is partly because analysis is often limited to in-silico gene panels, coding regions or focused on SNVs. We test the diagnostic yield in a cohort of patients affected by neurodegenerative diseases (NDD) by exploiting the sequence variations detectable by WGS.

Material and Methods: We analyzed WGS data of 140 NDD patients enriched for a positive family history of the disease and/or early onset. Most of them tested negative for the main causative genes of the disease. We applied a pipeline that allows the comprehensive analysis of all types of variants, (GANgSTR, CNV kit and Delly), to classify SNVs (Intervar) according to ACMG and those with a possible impact on splicing (SpliceAI). Massive in vitro functional assays (MPRA and MAPSY) were performed to evaluate the possible impact of VUS on gene expression or splicing mechanisms.

Results: In 27/140 cases(19%) we identified causative SNVs in NDDs genes. For other 17 cases(12%), we identified pathogenic or likely pathogenic SNVs in genes unrelated to patients' phenotype. Six patients carried repeat expansions in disease-related genes. A 1.2 Mb deletion with unknown significance was found in an ALS patient. Massive in vitro functional analysis identified four variants affecting gene expression and 16 splicing variants whose pathogenic role is still to be demonstrated.

Conclusion: WGS combined with in vitro testing allowed us to identify mutations for 16/140 patients undetectable with WES confirming that WGS can be the first-line genetic test in routine clinical settings.

C.O.I. Disclosure:

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P16.039.B On flowcell library construction and spatially linked reads provide a novel genome sequencing paradigm to significantly increase the utility of short reads

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Background: In 2024, scientists at Illumina, informed us of a new capability in early testing: the ability to create adapted library molecules and clusters directly on the surface of a glass flowcell and to use the spatial location of the clusters to link reads that were likely from the same starting DNA molecule.

Material and Methods: We identified 40 samples that we thought would exercise the capabilities of this new technology in our hands. These samples comprised blood and saliva-derived human samples, some with known clinically meaningful challenging variants, as well as 9 cell line derived DNAs with well curated truth data. Samples were prepared with or without a high molecular weight extraction. After dilution to the appropriate loading concentrations, DNA was loaded onto a NovaSeq X 10B flowcell with no library construction.

Results: We observed >90% of genes being fully phased and contig N50s of ~24 Mbp in high molecular weight samples. We observed better performance when the DNA was extracted with high molecular weight methods but still saw performance gains in samples that had been extracted with standard protocols. All 17 clinically meaningful variants in patient samples were found.

Conclusion: This method demonstrates significant improvements over standard short read sequencing, with low input requirements and almost no sample preparation requirements. We report here our findings, our thoughts on where this technology could be applied, current limitations, and considerations for what the final product that will be made available to the community might be.

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P16.040.C TRIXi: A high-resolution method for detecting skewed X chromosome inactivation

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Females have a higher risk for autoimmune disease and lower risk of mortality from infectious disease than males. While sex steroid hormones do affect immune responses, genetic and epigenetic factors, such as X-chromosome inactivation (XCI), also play critical roles. During embryogenesis, one X chromosome in each female (XX) cell is randomly silenced, resulting in a mosaic of cells with either the paternal (Xp) or maternal (Xm) X chromosome inactivated. However, skewed XCI (sXCI), where one parental X is preferentially silenced, can impact phenotypic variability and disease severity of X-linked disorders in females.Despite its significance, our understanding of sXCI in humans remains

limited. This gap in our knowledge primarily arises from the limitations in detecting sXCI, which continues to depend on methods like the HUMARA assay that lack sensitivity and precision. We introduce TRIXi (Tandem Repeat-based Identification of X-chromosome Inactivation), a novel high-resolution, cost-effective approach that assays XCI at multiple X-linked loci in a single reaction. Having validated the sensitivity and specificity of TRIXi, we assayed sXCI in over 1,000 healthy newborns (via the ABIS biobank) and revealed that extreme skewing of XCI is common (1–3%) at birth, and that constitutional skewing is stably maintained throughout childhood. Our findings are of importance for understanding the likely penetrance of X-linked disorders in human females and for our understanding of the frequency and dynamics of clonal hematopoiesis.TRIXi provides a simple, cheap and robust approach for studying sXCI and its role in female health.

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P16.041.D Reclassification of VUS using recommendations specified for sarcomeric genes in hypertrophic cardiomyopathy: resolution rate and technical considerations

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Background: Genetic testing most often provides a molecular diagnosis in hypertrophic cardiomyopathy (HCM). However, the presence of VUS remains a critical challenge, complicating the management of patients and their families. In April 2024, the ClinGen Cardiomyopathy Variant Curation Expert Panel adapted the ACMG/AMP guidelines for sarcomeric core genes (*MYH7,-MYBPC3,TNNI3,TNNT2,TPM1,ACTC1,MYL2,MYL3*), providing an opportunity to refine the classification of VUS.

Material and Methods: This study focused on the re-evaluation of 72 VUS originally identified in these genes in HCM patients tested between 2017–2024. Each variant was reassessed by multiple analysts, incorporating the most recent annotation data.

Results: Applying the gene-specific guidelines, we reclassified 18.1% of original VUS. The genes that benefited most were *TNNT2, MYL3, MYBPC3, MYH7*. Specifically, 92.3% of variants was downgraded to benignity (involving 17 patients) and the remaining 7.7% was upgraded to pathogenicity (affecting one patient). In terms of application rate, the leading criteria were population allele frequency (PM2 = 54%, BS1 = 15%), bioinformatic prediction (PP3 = 46%, BP4 = 24%) and definition of specific critical regions per gene (PM1 = 22%). However, most codes suffer from the lack of evidence (segregation data, functional and case-control studies). In addition to combining rules, the scoring system was used significantly (46.1%). Finally, 13.9% of VUS, still thus reported in Clinvar, were reclassified and the mean time to reclassification was 70.9 months.

Conclusion: These gene-specific rules reduce the number of VUS and standardise the interpretation of genetic variants in HCM, improving diagnostic yield and guiding patient management. However, further investigation and continuous updating of guidelines should be encouraged.

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P16.042.A Oxford Nanopore Technology (ONT) as an All-in-One Approach in the Diagnosis of Beckwith-Wiedemann Syndrome (BWS)

Federico Rondot[1], *Federica Centofanti*[2], *Luca Angheben*[3], *Matteo Paini*[3], *Federico Fontana*[3], *Niccolò Rossi*[3], *Flavia Cerrato*[4], *Laura Pignata*[4], *Giada Carta*[3], *Chiara Leso*[5], *Barbara Pasini*[1,6], *Alessandro Mussa*[7,8], *Andrea Riccio*[4,9], *Giovanni Battista Ferrero*[10], *Massimo Delledonne*[3], *Alfredo Brusco*[11,1]

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Background: Beckwith-Wiedemann syndrome (BWS) is a genetic disorder caused by imprinting defects at the 11p15.5 locus. Traditional molecular diagnosis involves multi-step procedures including methylation analysis of Imprinting Centers 1 (IC1) and 2 (IC2), detection of Copy Number Variants (CNVs), Uniparental Isodisomy Paternal (pUPD), and sequencing of CDKN1C. These workflows are resource-intensive and time-consuming.

Material and Methods: ONT-based approaches- Adaptive Sampling with MinION or PromethION (P2), and Genome Sequencing (GS) with P2 - were applied on three clinically and molecularly diagnosed BWS patient samples (IC2 defect, and mosaic pUPD). Performance metrics included SNP, indel, and CNV detection using simplex, simplex corrected with Herro (AI-based tool), and duplex calling aligned on hg38 and T2T assembly.

Results: ONT-AS confirmed all prior molecular diagnoses and showed high sensitivity in detecting pUPD mosaicisms. In terms of Gigabase output, GS-P2 outperformed AS-P2, producing an average of 115.6 Gb vs. 108.3 Gb, while AS-MinION averaged 14.7 Gb. AS-P2, aligned to hg38 and T2T assemblies, achieved superior coverage in regions of interest (ROIs: 167x vs. 168x), IC2 (161x vs. 162x), and IC1 (42x vs. 76.3) compared to other methods. Herro-corrected simplex and duplex calling enhanced SNP and indel detection compared to traditional simplex methods.

Conclusion: AS-ONT is a novel, efficient, and cost-effective method for BWS molecular diagnosis, allowing simultaneous analysis of methylation, CNVs, and sequence variations. Our results also suggest that AS-ONT is an effective method in the identification of pUPD mosaicisms. The integration of this approach into routine diagnostics could transform the landscape of imprinting disorder diagnostics.

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P16.043.B Using genome sequencing to improve diagnostic yield for gene panels

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Background: Genome sequencing (GS) is increasingly used as a first-tier test in patient care, with an 8–10% diagnostic yield increase in undiagnosed patients after exome sequencing (ES). Importantly, the exact gain varies by disease. Decreasing costs for GS have accelerated its wider clinical adoption. We evaluated the clinical utility of GS backbone for disease-specific gene panels vs. current ES approach.

Material and Methods: Diseases were categorized into 18 distinct groups and subgroups and were evaluated using genetic data from publicly available databases and our internal database according to defined criteria: 1) types of disease-causing genetic variation (SNV, CNV, repeat expansion, SV, deep-intronic variants, complex genetic events,⋯); 2) ability to detect such variants in ES vs. GS; and 3) groups with diseases caused by pathogenic variants in genes/regions not usually targeted in ES, but covered in GS (e.g. non-coding genes). Clinical utility of GS for each group was determined as the increased sensitivity to detect variants missed by ES and auxiliary assays not being necessary to complement panel design.

Results: Of the defined groups and subgroups, neurological and especially neuromuscular, neurodevelopmental disorders, and reproductive/carrier screening benefit most from GS. Conversely, bone disorders and dermatology benefit the least. Disease group data will be presented in detail per gene and variant type.

Conclusion: We suggest that GS be considered as the backbone of choice for disease-specific gene panels where both diagnostic yield and cost efficiency can be maximized. Furthermore, GS enables re-analysis of genomic data as scientific knowledge advances and new evidence arises.

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P16.044.C Standardizing the gene composition of the clinical exome: a semi-automated bioinformatics framework for evaluating gene-phenotype associations

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Background: The rapid and cost-effective identification of disease-causing variants using whole-exome sequencing (WES) has driven its widespread use in clinical diagnostics, facilitating the concurrent analysis of multiple disease-causing genes. While disease-specific multigene panels are commonly used, a more comprehensive, disorder-agnostic approach may be preferred, incorporating all genes associated with Mendelian diseases, known as the clinical exome (CE). Given the large number of genes comprising the CE, and novel gene-phenotype relationships being continuously discovered, a bioinformatics approach is essential for efficiently creating and updating this panel.

Methodology: An algorithm was developed to generate a disease-associated and clinically relevant genes' list, using an in-house knowledgebase integrating information from several databases such as OMIM, OrphaNet, HGMD, HGNC, NCBI and SFARI. Medical and clinical laboratory geneticists then curate this list to establish a CE panel.

Results: Of 13,076 clinically relevant genes with an HGNC entry, 5,653 were directly added to the CE, and the rest further analyzed. By examining the number of gene-phenotype pairs and HGMD-described disease-causing variants, 3,347 genes were discarded, 3,819 genes included, and the remaining 257 genes subjected to a final manual curation step. Further inclusion of mitochondrial loci and 23 lab-curated genes resulted in a CE panel containing 9,326 genes. These were then further classified according to their analytical effectiveness, by considering their WES-based horizontal coverage.

Conclusion: Optimizing bioinformatic approaches is critical in the fast-evolving clinical diagnostics space. This work presents a new framework to streamline the creation of such complex gene panels, ensuring they remain up-to-date and clinically relevant.

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P16.045.D Learnings from five years of External Quality Assessment (EQA) for porphyrias

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Background: Porphyrias (POR) represent eight genetic disorders characterized by the accumulation of porphyrins or their precursors. Affected individuals develop severe cutaneous photosensitivity and/or suffer from attacks of severe abdominal pain. Proficient diagnosis is required for effective clinical management. EMQN established a global EQA scheme in 2005, aimed at ensuring the proficiency of genetic testing for POR.

Material and Methods: Participants received three or four DNA samples with corresponding mock clinical referrals for testing. Participants returned clinical reports which were assessed for genotyping, interpretation, and accuracy (maximum score per category = 2.00). Participant performance was analysed over a 5-year period (2020–2024) to establish trends and understand the impact on reporting quality from continued EQA provision.

Results: Ninety-six participations have been assessed from 32 laboratories in 17 different countries. Genotyping quality was consistently excellent with an average score of 1.93/2.00. However, several laboratories missed a known pathogenic intronic *FECH* variant: NC_000018.10(NM_000140.5):c.315-48T>C p.?. Interpretation quality was also very good, averaging 1.83/2.00 over the period surveyed. In 2023, interpretation performance dropped to 1.63/2.00, due to 55% of laboratories failing to interpret the genotype result in the context of the clinical referral.

Conclusion: These results show POR testing services are consistently of a good standard. However, improvements could

be made in ensuring the scope of testing for rare causes (i.e.-*FECH*) and improving interpretation to ensure reports are informative for the recipient. We anticipate the continued provision of this EQA is crucial for improving testing and reporting standards for POR.

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P16.046.A Pioneering study on dd-cfDNA as a rejection biomarker in Kazakhstani transplant population

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"University Medical Center" Corporate Fund, Astana, Kazakhstan

Background: Accurate monitoring of transplant recipients is essential for early rejection detection. Donor-derived cell-free DNA (dd-cfDNA) is a promising biomarker, with digital PCR (dPCR) offering high sensitivity. This study, the first of its kind in Kazakhstan, is also the first to investigate dd-cfDNA using dPCR and Y-chromosome fragment analysis in kidney and heart transplant recipients.

Material and Methods: The prospective study recruited 40 adult and 15 pediatric kidney transplant pairs, along with 30 heart transplant pairs, at the "University Medical Center" CF and the National Research Oncology Center in Astana. Among the recipients, 10 females received organs frommale donors, enabling dd-cfDNA detection through Y-chromosome markers.

Results: Results demonstrated that dPCR accurately quantifies dd-cfDNA, with elevated levels correlating with rejection episodes. This supports dd-cfDNA potential as a diagnostic tool for transplant monitoring. Monitoring of heart transplant recipients revealed an increased level of dd-cfDNA in one patient with clinical signs of allograft rejection. This approach also demonstrated a significantly higher level of dd-cfDNA in a kidney transplant recipient who received an organ from a deceased donor compared to those who received organs from living donors during the first two weeks post-transplantation.

Conclusion: These findings mark the first use of dd-cfDNA as a predictor of transplant rejection in Kazakhstan. Further studies are needed to confirm these results and optimize clinical applications.

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P16.047.B Introducing GENie: A two-year journey of discovery and competency

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Background: As variant classification guidelines and cytogenetic nomenclature are constantly evolving the need for individuals to demonstrate competency for variant classification and report genomic test results following current guidelines has increased. The development of the Genomics Individual Education (GENie) platform is being used to support individual competency demonstration and promote workforce education.

Material and Methods: The GENie platform contains modules for classifying single nucleotide variants (SNVs), copy number variants (CNVs), use of HGVS, and ISCN. The ISCN module provides education and competency assessment for using ISCN 2024 for Constitutional Karyotyping, Neoplasia Karyotyping, Constitutional FISH, and Neoplasia FISH at basic, advanced, and complex levels. The variant classification modules present cases for participants to determine the pathogenic classification and submit supporting evidence. Real-time assessment is provided.

Results: 1,873 individuals have participated in all six nomenclature scenarios, HGVS/ISCN, and completed five-seven cases in each scenario. The expected HGVS/ISCN was submitted by 75% and 60% of participants, respectively. Additionally, there were 1,273 participations for classifications of SNV/CNV, whereby a random set of three-six variants was provided for classification. The expected classifications were submitted by approximately 1,000 participants. All scenarios were generated from a vast bank of cases. The range of these scenarios will be presented.

Conclusion: In the last two years, the GENie platform has been a promising solution for supporting variant classification and nomenclature interpretation practices which is already used by thousands of individuals. By using GENie, individuals can gain the necessary knowledge and skills, which will help them to maintain their competency.

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P16.048.C Performance comparison of RNA-seq kits for accurate gene expression profiling across different input levels

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Background: RNA sequencing (RNA-seq) is a powerful technique for analyzing gene expression and transcript structure. Different kits vary in their methodologies, efficiency, and ability to capture low-abundance transcripts, which can significantly influence data interpretations. In this study, we compare multiple RNA-seq kits to provide researchers with insights into selecting the most appropriate platform for their specific experimental needs.

Material and Methods: Three commercially available RNA-seq kits, Lexogen's QuantSeq 3'mRNA-Seq V2 FWD Kit, New England Biolabs' NEBNext Ultra II Directional RNA Library Prep Kit, and Watchmaker Genomics' mRNA Library Prep Kit, were evaluated using a human **reference RNA and fibroblast-derived RNA**. Libraries were prepared with input amounts of **2.5, 10, 50, 100, and 200 ng**, following each kit's protocol. Quality was assessed using metrics like total sequence, alignment rates, transcript coverage, and differential expression.

Results: All kits produced similar sequencing output and alignment rates. However, the Watchmaker Genomics kit showed better consistency, particularly with lower RNA input. The Lexogen kit yielded fewer protein-coding reads and identified fewer differentially expressed genes compared to the full-length RNA-seq kits from New England Biolabs and Watchmaker Genomics.

Conclusion: In conclusion, while all RNA-seq kits had similar sequencing and alignment results, full-length mRNA sequencing applications showed better consistency, with the Watchmaker Genomics kit showing the highest consistency across different input amounts, and a higher sensitivity in differential expression analysis. Our findings emphasize the importance of kit selection based on input type and research goals and suggest that low-pass full-length mRNASeq outperforms 3'mRNASeq for expression profiling applications.

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P16.049.D Are cytogenomic competencies declining?

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Background: GenQA has provided external quality assessment (EQA) worldwide for cytogenomic testing for over 40 years. Accurate cytogenomic skills are assessed for analytical competence and the cytogenomic knowledge required to correctly interpret the result in the context of the provided case scenario. Cytogenomic training has changed dramatically over the years, with a greater focus on molecular technologies and a move away from traditional cytogenetics. The result is a decrease in the number of trained Cytogeneticists able to deliver accurate analysis and interpretation of chromosomes. GenQA EQA performance therefore provides an opportunity to examine trends over a long period.

Methods: Examination of the cytogenomic EQAs was undertaken. Constitutional EQAs included pre- and postnatal karyotyping, and pregnancy loss G-banding. Review of the haematology EQAs incorporated ALL, CLL, myeloid, lymphoma and mature B&T G-banding. Analytical errors and poor interpretation were addressed.

Results: Incorrect karyotypes and interpretation of cytogenetic abnormalities have been reported in constitutional EQAs, including multiple analytical errors for reporting the incorrect karyotypic sex. For haematological malignancies recurrent interpretation errors include incorrect diagnosis, incorrect prognosis, providing outdated therapeutic information, and failing to include a WHO/ICC classification. There are also errors regarding the counting of chromosome abnormalities (required for determining prognosis). The number and type of errors will be examined in detail.

Conclusion: Numerous errors and recurrent trends have been identified through EQA both for analysis and interpretation. From an EQA perspective, the increasing number of errors demonstrates the decline in cytogenomic competence.

C.O.I. Disclosure:

M.J. Sales: None. **F. Oswald:** None. **M. Tabiner:** None. **S. Deans:** None.

P16.050.A Diagnostic evaluation of Oxford Nanopore Technology in STR screening for neurological diseases

V. L. Race[1], *E. Souche*[2], *S. Meynants*[2], *A. Van Eesbeeck*[1], *L. Budts*[1], *S. Smekens*[1], *M. Taffeiren*[1], *T. Luyten*[1], *R. Vandensande*[1], *L. Keldermans*[1], *A. Goovaerts*[1], *S. Vermeer*[1], *J. Vermeesch*[3];

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Background: Neurogenetic diagnostics standard of care consists of short read whole exome sequencing (WES). While WES performs well for the detection of single nucleotide variants and indels, it is less accurate for the detection of short tandem repeats (STRs). This is definitely a shortcoming in neurogenetic diagnostics since STRs are an important cause of neurological disease such as ataxia, amyotrophic lateral sclerosis/ frontotemporal dementia, myotonic dystrophy. WES is thus currently complemented with a combination of specific repeat-primed PCRs.

Material and Methods: In order to evaluate whether long read sequencing could replace our standard of care, Oxford Nanopore Technology (ONT) sequencing was performed on six patients with known STR expansions (SCA2(ATXN2), CANVAS (RFC1), SCA27B (FGF14), Friedreich (FXN), ALS (C9orf72) and Steinert (DMPK)).

Results: Using a standard DNA extraction, all STRs could be detected by ONT sequencing and the repeat length was concordant with diagnostic results. In addition, new data are also generated from the methylome.

Conclusion: ONT is thus promising since (1) the same DNA extraction can be used for WES and ONT STR analysis while other technologies such as optical genome mapping require a specific DNA extraction methods; (2) the size estimate of all STRs is accurate preventing the need to work with arbitrary cut offs; (3) eventual repeat interruptions can be detected; (4) the methylation status is available, which is not the case with the standard diagnostic tests. The use of ONT for STR screening could improve the diagnosis of neurogenetic diseases and have an impact on patient follow-up/therapy.

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P16.051.B A *BRCA2* copy number variant called as a simple duplication turned out to be a rare complex rearrangement

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Background: To identify the underlying pathogenic variant in families with breast and ovarian cancer, germline analysis of potentially affected genes is standard practice. The list of relevant genes has been defined by the German Consortium for Familial Breast and Ovarian Cancer.

Material and Methods: Peripheral blood DNA was enriched using an exon-focused gene panel. Sequencing was performed on an Illumina NextSeq 2000. Read mapping and variant calling was performed in the cloud-based Varvis® software.

Results: In a case of familial breast and ovarian cancer, a simple duplication at the end of exon 11 was reported. Visual inspection of the mapped and soft-clipped reads in the Integrative Genomics Viewer (IGV) revealed a deletion and a translocation by colour coding. Reconstruction of the split reads indicated that the duplicated region was translocated within the exon, with an additional deletion of two nucleotides and insertion of seven nucleotides. Breakpoints of the putative complex rearrangement were verified by specific PCR products followed by sequencing. Theoretically, this should lead to differential splicing, but this could not be accurately predicted. To determine the consequences at the RNA level, cDNA sequencing was performed to apply the correct ACMG criteria and finally to determine the pathogenicity of the variant.

Conclusion: Although duplicated material is usually inserted in tandem next to the original region, there are rare cases of complex rearrangements. These can and should be detected by careful inspection of the mapped reads. The consequences of these rearrangements generally need to be addressed by RNA sequencing.

C.O.I. Disclosure:

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P16.052.C A phenotype-based approach for prioritizing ASD genetic variants from whole-exome sequencing

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Background: Current genetic diagnostic approaches of autism spectrum disorder (ASD) have a relatively low yield of 3%–28%, thus underscoring the need for new methods. Here, we present a novel phenotype-driven approach to identify and prioritize ASD genetic variants.

Material and Methods: We studied phenotype and whole-exome sequencing (WES) data from 115 children with ASD from the Azrieli National Center for Autism and Neurodevelopment Research (ANCAN) database. We used the Human Phenotype Ontology (HPO) nomenclature to group all phenotypes into 41 broad phenotype categories. Then, we used these categories to characterize each participant with an Observed Phenotype Vector (OPV), and each gene in the HPO database with an Expected Phenotype Vector (EPV). Finally, we assessed the EPV-OPV concordance for each detected gene-disrupting variant (GDV) in the WES data.

Results: Overall, 232 HPO terms grouped into 41 broad phenotype categories were identified in our sample. High-confidence ASD genes (from the SFARI database) had significantly higher counts of expected phenotypes than non-ASD genes (15.46 ± 6.6 vs. 7.26 ± 5.96, $p < 2.2e\text{-}16$), hence underscoring a link between the 41 phenotype categories to ASD genes. Of the 593 identified GDVs, 35 showed significant EPV-OPV concordance ($p < 0.05$). Of these 11 variants were found in known ASD genes while another 24 were identified in 22 potentially novel ASD genes.

Conclusion: This phenotype-driven approach shows potential for identifying novel genetic variants linked to ASD, thus substantially increasing the genetic diagnostic yield of the disorder.

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P16.053.D Detecting situations that may compromise non-invasive prenatal diagnostic of monogenic diseases

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Background: Non-invasive prenatal testing for monogenic disorders (NIPD-M) is becoming increasingly popular to detect the presence of known parental mutations in the fetus, without risking miscarriage. However, as the method heavily relies on determining the haplotype balance in cell-free DNA circulating in maternal plasma, it can potentially be confused by various situations, such as aneuploidy of the target chromosome or unrecognized (possibly vanishing) twin pregnancies.

Material and Methods: By mixing DNA from male donors, we generated composite samples with various numbers of different X chromosomes, to simulate a small amount of fetal DNA in maternal DNA (aiming for a 20% fetal fraction). We simulated various challenging situations, like twin pregnancy or aneuploidies, homogeneous or in mosaic.

Results: Examination of the allelic frequency of 5 types of SNPs located on the X chromosome allowed straightforward detection of most abnormal situations, down to a low level of mosaicism. The exceptions were maternal trisomies, for which we had to develop an additional strategy: We informatically reduced fetal fraction by "diluting" data from simulated plasma samples with maternal data and plotted allelic frequency of type-4 (maternal) SNPs against fetal fraction. The linear regression slope proved a powerful indicator to detect maternal trisomies, including mosaics.

Conclusion: We introduce a metric allowing, without additional wet-lab investigations, to reliably detect the presence of anomalies that may compromise NIPD-M results. We show that it can detect twin pregnancies, paternal or maternal trisomies, monosomies and uniparental disomies, down to low fetal fractions and low percentages of mosaicism.

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J. Blouin: None. **C. Rieubland:** None. **N. Thierry:** None.

P16.054.A Beyond compliance: how external quality assessment drives laboratory improvement over time

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Background: External Quality Assessment (EQA) is a crucial tool for monitoring and improving laboratory performance, ensuring the accuracy and reliability of genetic testing. However, the long-term impact of sustained participation in EQA schemes on laboratory performance remains underexplored.

Material and Methods: This study examines laboratory performance trends across EQA schemes over a 10-year period, with a focus on marking changes and the occurrence of poor performance—defined as errors with the potential to cause patient harm. Key performance metrics, including genotyping accuracy, interpretation quality, and clerical precision, were evaluated across multiple EQA cycles. Statistical analysis was conducted to identify patterns in poor and persistent poor performance (characterised as consecutive years of substandard results) and to assess their relationship with the frequency of EQA participation.

Results: A trend emerged, showing that sustained participation in EQA leads to improved laboratory performance. Laboratories with higher participation rates demonstrated a reduction in critical genotyping and interpretation errors. Regular participants showed a marked decrease in poor performance rates and overall accuracy improved compared to first-time participants, with the most notable gains observed in interpretation scores. Despite these improvements, a subset of laboratories continues to experience performance fluctuations, underscoring the need for targeted interventions.

Conclusion: EQA plays a pivotal role in driving continuous improvement in laboratory performance, reducing errors, and enhancing the reliability of genetic testing. While these findings

reinforce the importance of sustained EQA participation, they also suggest that more tailored support strategies may be required for laboratories struggling with persistent poor performance.

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P16.055.B DNA sample tracking for diagnostic exome sequencing - a 1-year experience

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Background: Whole exome sequencing (WES) and other massively parallel sequencing-based tests are well established in clinical diagnostics. However, as these workflows require numerous wet- and dry-lab steps, each increasing the probability of errors, DNA sample tracking throughout the workflow is recommended to identify sample swaps. Here, we report the results of WES sample tracking during a 1-year period in an academic hospital.

Material and Methods: Library preparation for WES and sample tracking was performed in parallel per 96 samples using the KAPA HyperExome V1 (Roche) and the Human Sample ID (HSID, pxlence) kits, respectively. Next, 192 WES and 192 HSID libraries were sequenced together on a NovaSeq 6000 S4 flow cell (Illumina), followed by a Nextflow-based pipeline applying CrosscheckFingerprints (Picard) to compare 43 autosomal SNPs and SampleGender (ngs-bits) to evaluate the X/Y ratio, coverage of the SRY gene and the fraction of heterozygous SNP's on X/Y.

Results: This poster will summarize results of sample tracking performed on >7,500 WES patient samples through HSID kit genotyping of 49 exonic SNPs. Preliminary analysis on a subset of samples revealed an 82.29% index uniformity. On-target coverage is high with 97.92% of samples having over 90% of reads aligning to the regions of interest. Marker coverage uniformity shows that in over 74.48% of samples, over 75% of the autosomal markers attain a coverage within 2-fold of the median coverage.

Conclusion: The HSID kit is an easy-to-use and cost-effective approach for DNA sample tracking in massively parallel sequencing workflows, ensuring quality in accredited laboratory settings.

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P16.057.D FrOG - The French OncoGenetics database

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FrOG is the reference database of genetic variants identified in oncogenetic laboratories in France, the interpretation of these variant is validated by various curation groups of the Groupe Génétique et Cancer (GGC). The mission of FrOG is to guarantee and disseminate a clinically relevant interpretation, harmonised at national level, in order to improve the care of families. FrOG is governed by a consortium agreement, coordinated by UNICANCER, that brings together 24 University Hospitals and Cancer Center healthcare institutions. The laboratories of these institutions, in collaboration with the Groupe Génétique et Cancer (GGC), have an IT tool for collecting, curating and sharing patient data via a website (frog-db.fr). FrOG complies with the requirements of the General Data Protection Regulation (GDPR). A patient information leaflet, validated by the GGC Board, has been distributed to oncogenetic consultations to harmonise discussions on the benefits and even the need for the FrOG programme and to explain patients' rights. An information video can be found at https://youtu.be/tZs6y324GSk?feature=shared. FrOG benefits from an infrastructure hosted by an accredited health data host. New variants and associated phenotypic data are deposited by authorised contributors in pseudo-anonymised form in a secure repository. FrOG-db currently offers interpretation of 17563 variants of 33 genes identified in 52107 patients. FrOG is consulted several hundred times a day as part of molecular diagnostics. This academic project now meets the various regulatory requirements in force and has become an essential tool for diagnosing patients in the French oncogenetics network.

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P16.058.A Combinatorial DNA-Pools targeted-Sequencing as a robust cost-effective method to detect rare variants: analysis strategy and application to dilated cardiomyopathy genetic diagnosis

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Background: We developed a cost-effective NGS method based on combinatorial DNA pooling to efficiently detect rare variants. The originality of our approach lies in the development of a specific data analysis method that accurately discriminates true rare variants from false positives within pools. The experimental workflow and data analysis were validated by comparison with standard simplex sequencing technology. This method was applied to the genetic diagnosis of dilated cardiomyopathy (DCM).

Methods: 96 non-indexed 8-DNA pools were constituted from 384 DNAs, each being included in a unique pair of concordant pools (sequenced twice though). Exonic regions of a cardiomyopathy gene panel were captured and sequenced by NGS at 500X per pool. Variant calling was performed using Freebayes algorithm, and the resulting QUAL value was compared between variants detected in concordant pools (true variants) and non-concordant pools (false positives) to accurately identify true variants. To benchmark performance, 50 DNA samples were sequenced using standard simplex NGS.

Results: Freebayes parameters QUAL = 12 was found to be discriminative between false-positives and true variants. Using this threshold, our pool-sequencing method successfully detected 96% of rare variants identified in DNA-simplex. Applied to 384 DCM patients, this approach identified 100 pathogenic ACMG class 4 and 5 variants (26%).

Conclusion: We report an innovative, cost-efficient (4-fold cost reduction) NGS pooling method with a dedicated variant analysis strategy that enables accurate detection of rare variants. This approach might be applied more broadly for cost-effective genetic diagnosis in various rare diseases.

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P16.059.B Is there anything in the swamp? A credibility assessment of biologic signals from non-secreted low-abundance proteins measured in human plasma

Roberto Spada[1], *Jackie Dennis*[2], *Joe Gogain*[2], *Clare Paterson*[2], *Jessica Kuzma*[2], *Joel Wommack*[2], *Emma Troth*[2], *Stephen A. Williams*[2], *EPIC Study Collaborators*[3]

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Background: Proteomic multiplexing has expanded and now includes measurement of thousands of non-secreted low-abundance proteins, yet the biological significance of this fraction of the proteome has been questioned. We present here methods to evaluate this question by determining: 1) if proteogenomic associations are present in the non-secreted, low abundance fraction, 2) if this fraction contains other biologically relevant information.

Materials and Methods: We surveyed peer-reviewed publications to determine the number of cis-pQTLs identified on the SomaScan® Assay. Of those, we calculated the proportion of cis-pQTLs in the non-secreted, low-abundance fraction of the proteome. We used data from~15,000 participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study assayed on SomaScan 7K to determine what proportion of the significant protein biomarkers associated with incident cancers were in the non-secreted low-abundance fraction.

Results: Roughly 2,800 cis-pQTLs identified by SomaScan Assay have been reported in peer-reviewed publications. There are ~5,000 non-secreted low-abundance proteins on the SomaScan 7K menu; 30% (n = 1494) contain a cis-pQTL vs. ~60% of secreted proteins. Of the statistically significant biomarkers for cancer risk, 68% and 60% respectively were found in the non-secreted low-abundance fraction.

Conclusion: Non-secreted proteins comprise a greater proportion of the proteome than known secreted proteins. Despite unclear mechanisms relating to their presence in plasma, they contain important biologic information. Reassurance of correct protein binding in this fraction arises directly from cis-pQTLs and from biomarkers of future cancer diagnosis. Proteomic technologies that can be scaled while maintaining sensitivity and precision will continue to yield useful discoveries.

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P16.060.C Detection of Recombinant Alleles in *GBA*Gene Using Short-Read NGS: An Accessible Approach in Routine Genetic Analysis

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Background: Short-read next-generation sequencing (NGS) is widely used in genetic diagnostics due to its low error rate, cost-effectiveness, and accessibility. However, it is generally considered inadequate for detecting complex genomic rearrangements, particularly in genes like *GBA*, which has a highly homologous pseudogene. Long-read NGS is often preferred for identifying such structural variants, but its adoption remains limited. Exonic GBA Sequencing, MLPA, and Whole-Gene GBA Sequencing are performed comprehensively in our diagnostic algorithm. Identifying complex rearrangements is particularly crucial in unresolved cases. This approach enhances our ability to clarify previously unsolved cases.

Material and Methods: we report the detection of recombinant alleles using short-read NGS, relying primarily on IGV visualization, which requires expert interpretation rather than additional computational tools. Since September 2022, 1,790 individuals were analyzed for Gaucher disease. Findings were validated using long-read and Sanger sequencing.

Results: Among the 1,790 individuals analyzed, 373 (20.8%) carried at least one pathogenic or likely pathogenic variant, including 143 patients and 230 carriers. Notably, 17 of these variants (4.6%) were recombinant alleles identified in nine patients and six carriers.

Conclusion: Our analysis revealed that recombinant reads are often misaligned in standard whole-genome alignments, with each recombinant allele exhibiting a distinct fingerprint pattern in either GBAP1 or GBA gene. Our findings challenge the assumption that recombinant alleles in GBA go undetected in routine short-read NGS. We demonstrate that short-read NGS, when combined with careful IGV visualization by an experienced team, can successfully identify complex rearrangements, offering a more accessible and cost-effective alternative for routine diagnostics.

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P16.062.A Interesting cases as demonstrator for a one size fits all solution in molecular diagnostics

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Background: While microarray is the preferred method for detecting copy number variation (CNVs), next-generation sequencing offers greater versatility, detecting single nucleotide variants (SNVs), indels, structural variants and CNVs with high resolution (minimum detectable size of 50 kb in microarray vs. single exon level resolution in NGS) and sensitivity, as well as viral infection specifically in a prenatal setting.

Material and Methods: We aimed to create a platform capable of identifying all type of genetic variations. Therefore, we developed a customized whole exome enrichment method incorporating protein coding regions, over 46,000 non-coding clinically relevant regions, a genome-wide CNV backbone, and targets for certain pathogens that are known to potentially lead to prenatal abnormalities.

Results: Here we summarize the genetic findings detected by whole exome sequencing (WES) in three patients with suspected complex genetic disorder. Genetic variants comprised (i) an unbalanced translocation (breakpoints in 3p25.3 and 5p15.32), (ii) a large de novo structural variant of the X chromosome including a terminal deletion of 1 Mb at Xp22.33 and a terminal gain of 3.2 Mb at Xq28, and (iii) a compound heterozygous identification of a small CNV (deletion of exons 3–5) and an SNV in the *PIGG* gene (4p16.3). In another case, we were able to detect Parvovirus B19 DNA in a fetus with hydrops fetalis.

Conclusion: Our exome design, combined with a robust CNV pipeline and pathogen targets, enables the detection of not only SNVs but also CNVs, structural rearrangements, and viral infections in WES.

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P16.063.B Challenges and Pitfalls in diagnosing twins with discordant BWS phenotype

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Background: Beckwith-Wiedemann syndrome (BWS) is the most common genetic cause of overgrowth, occurring in about 1 in 10,000 live births. It results from imprinting defects in the 11p15 region and presents with a broad range of features, such as hemihypertrophy, macroglossia, abdominal wall abnormalities, hypoglycemia, and an increased risk of embryonal cancers.[1,2] Despite the use of a clinical score to guide molecular diagnosis, BWS shows considerable phenotypic variability, even among individuals with the same genetic defect, as appreciated in monozygotic twins.[3,4]

Material and Methods: Two monochorionic-diamniotic female twins, born from spontaneous pregnancy, with discordant clinical phenotype were assessed at our Rare Disease Unit. The proband, the first-born twin, was tested on suspicion of Silver-Russell syndrome due to intrauterine growth restriction and feedingdifficulties. The second twin showed physiological growth pattern and neurodevelopment with no sign of BWS. Both twins were investigated with multiple ligation-dependent probe amplification (MS-MLPA) of the11p15 region, and the first twin was tested with MS-MLPA in multiple imprinted loci.

Results: Both twins we found sharing the same hypomethylation pattern of the KvDMR/IC2 region. MS-MLPA in multiple imprinted loci of the first twin was negative.

Conclusion: Our report aims to support clinicians facing challenges and pitfalls in diagnosing and managing BWS in twins with discordant phenotype. While waiting for a shared diagnostic algorithm, based on a scoring system, to deal with unexpected results to start the appropriate oncologic surveillance programme, we recommend to always test both twins, exercising caution in data interpretation.[5]

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P16.064.C A comprehensive benchmark study of multiple library amplification solutions for NGS

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Background: Precision oncology leverages next-generation sequencing (NGS) to guide decisions based on an individual's genetic profile. As the demand for these applications increases, minimizing errors and biases introduced during library preparation and sequencing becomes essential. PCR amplification is a significant source of potential errors in NGS library preparation, with single-base substitutions, insertions, and deletions (indels), as well as PCR-induced biases in DNA length and GC content, all contributing to data distortion and reduced assay sensitivity and specificity. Enhancing library amplification techniques, coupled with advancements in sequencing technology, will improve NGS assay reliability and performance.

Methods: This study evaluated several commercial library amplification systems, including two from Watchmaker Genomics: Equinox and a newly engineered library amplification mix under development. Using a UMI-powered NGS assay, we assessed base fidelity, indel rates in microsatellite sequences, chimeric reads, and GC-uniformity. Libraries were sequenced on multiple platforms, leveraging the high base quality scores from state-of-the-art sequencing chemistries for precise measurements of polymerase

fidelity. We also examined UMI family depth uniformity, a valuable indicator of template sequence bias and error-correction effectiveness. Additionally, we assess amplification size bias and both SPRI and streptavidin beads compatibility. Finally, we test the ability to generate high terminal library yield, which is important for hybridization capture workflows.

Results and Conclusions: Our findings show that while some products perform well on certain metrics, they underperform in others. Notably, Equinox—especially the newly engineered version—demonstrated consistent, robust performance across all relevant metrics, positioning it as a leading solution for accurate and unbiased library amplification.

C.O.I. Disclosure:

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P16.065.D Unexpected findings in *MECP2* during genetic workup for cardiological symptoms

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Background: A 39-year-old female presented with ventricular tachycardia and syncopes, raising suspicion of a hereditary cardiological disease.

Material and Methods: Singleton exome sequencing was performed, and variants were classified according to ACMG guidelines.

Results: A heterozygous frameshift variant in *MECP2* was identified: NM_004992.3:c.1269_1287del p.(Leu424Profs*52) and initially classified as likely pathogenic (PVS1, PM2_Supporting) according to ClinGen Rett and Angelman-like Disorders Expert Panel Specifications for *MECP2* (Version 3.0.0), although the patient showed no developmental or cognitive abnormalities, nor other symptoms of Rett syndrome. Subsequent targeted analysis of family members revealed that the ten-year-old son (hemizygous) and mother (heterozygous) carried the same variant. Neither displayed clinical symptoms related to *MECP2*-associated disorders, necessitating the reclassification of the variant as uncertain significant (PVS1, PM2_Supporting, BS2).

Conclusion: This raises questions about why this *MECP2* variant does not lead to symptoms of Rett syndrome. Explanations remain speculative unless functional studies may offer valuable insights. Truncating variants escaping nonsense-mediated decay are consistently reported in *MECP2* and have been observed in both Rett syndrome patients and occasionally in population databases (gnomAD), making clinical interpretation challenging. Incomplete penetrance in *MECP2*-related disorders has not been documented in literature. Given the established connection between *MECP2* and cardiac phenotypes, such as long QT syndrome, it is possible that the patient's symptoms are related to this variant, however, isolated cardiac abnormalities are typically not observed in patients with pathogenic *MECP2* variants. This case emphasizes the complexities of genetic counseling in similar cases and highlights the need for more nuanced, evidence-based discussions in variant interpretation.

C.O.I. Disclosure:

S. Chahrokh-Zadeh: None. **L. Frey:** None. **A. Schreiner:** None.

P16.066.A Cost-effective multiplex digital PCR quantification using universal Rainbow detection probes

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Background: While the latest generations of digital PCR instruments enable the quantification of 4 to 8 targets in a single reaction, the design, synthesis, and validation of target-specific probes is a bottleneck. Universal probes are not target-specific and can dramatically speed up the development of multiplex assays while keeping costs and turnaround times under control.

Material and Methods: A Rainbow probe (pxlence), a Rainbow primer (i.e., a tailed primer so that the amplicon gets recognized by the probe; pxlence), and a regular primer were used in asymmetric PCR to selectively amplify and quantify a target on 3 different digital PCR instruments (QX200, Digital LightCycler, and

QIAcuity) using the instrument-specific PCR master mix. For mutation analysis, double-mismatch allele-specific primers were designed according to Lefever et al., 2019 (Scientific Reports); all other applications used a modified primer previously successfully used with a DNA binding dye or target-specific probe.

Results: We will present various digital PCR applications in which Rainbow probes enable straightforward multiplex detection of targets on three different digital PCR instruments. These applications range from 2-plex HER2 copy number quantification in cell-free DNA from breast cancer patients to 6-plex gene copy number quantification in genomic DNA, 2-plex transcript quantification in extracellular vesicles, 5-plex gene expression analysis in differentiating induced pluripotent stem cells, and single nucleotide variant quantification using allele-specific primers (BRAF V600E, ALK F1174L).

Conclusion: The use of universal Rainbow detection probes enables cost-effective multiplexing with short turnaround times, speeding up assay development across a wide range of applications.

C.O.I. Disclosure:

G. Wils: A. Employment (full or part-time); Significant; pxlence. **E. Jacobs:** A. Employment (full or part-time); Significant; pxlence. **A. Declercq:** A. Employment (full or part-time); Significant; pxlence. **P.N. Ceulemans:** A. Employment (full or part-time); Significant; pxlence. **J. Hellemans:** F. Consultant/Advisory Board; Significant; pxlence. **F. Coppieters:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; pxlence. **S. Lefever:** F. Consultant/Advisory Board; Significant; pxlence. **J. Vandesompele:** F. Consultant/Advisory Board; Significant; pxlence.

P16.067.B Thalassemia panel: a novel diagnostic approach

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Thalassemia is one of the most heterogeneous diseases, affecting 5–7% of the global population. The most relevant forms are α- and β-thalassemia, caused by mutations in the HBA and HBB genes, respectively. Molecular diagnosis of thalassemia using conventional PCR-based DNA analysis has limitations due to the phenotypic variability and heterogeneity of mutations.

Methods: Next Generation Sequencing data obtained with Thalassemia panel kit (H1090, 4bases Italia), a CE-IVD amplicon-based kit, suggested a more accurate DNA analysis ensuring the identification of single nucleotide variants (SNVs) and the most common Copy Number Variants (CNVs) in a single test, achieving a gain in terms of time and accuracy. The kit validation was performed using 5 commercial standard samples (Coriell Institute), with known and emblematic genotypes: SEA heterozygous (het), FIL het, 3.7del homozygous, SEA-FIL, and Lepore-Baltimore het (3 samples were used as negative controls). Data analysis was conducted with 4eVAR, 4bases proprietary analysis platform, ensuring reliable results through a validated internal workflow.

Results: Thalassemia-causing CNVs represent an analytical challenge due to the overlap and difficulty in mutual discrimination with traditional analysis methods. Validation data showed that all expected mutations have been identified, demonstrating that combined coverage and GAP-PCR analysis helped identify and distinguish frequent overlapping CNVs, with SNVs detected through optimal coverage (>100x) of all panel targets.

Conclusion: The Thalassemia panel kit represents an accurate and fast diagnostic solution for precision medicine, enabling not only the evaluation of mutations related to a thalassemic clinical phenotype, but also family or retrospective studies focused on specific CNVs/SNVs.

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V. Barzon: A. Employment (full or part-time); Significant; 4bases SRL. **V. Favalli:** None. **S. Casali:** A. Employment (full or part-time); Significant; 4bases SRL.

P16.068.C A Framework for Efficient Variant Filtering in Whole Genome Sequencing for Improved Diagnostics

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Background: Rare genetic diseases present significant diagnostic challenges due to genetic diversity and limitations of current sequencing and analysis techniques. Exome Sequencing (ES), which focuses on coding regions, has a limited diagnostic yield of 25–35%. Whole Genome Sequencing (WGS), which includes non-coding regions, structural variants, and intronic sequences may offer higher diagnostic potential. However, the vast number of variants and insufficient annotation of non-coding regions in the global databases only lead to a modest increase in the additional diagnostic yield of WGS on top of ES variant analysis.

Material and Methods: This prospective study evaluated a new filtering method (referred to as smart filtering) on 100 patients with various phenotypes who previously had negative results from ES and conservative WGS analysis. The data was processed with the Geneyx Analysis platform for alignment and variant calling. Pre-filtering step was applied (referred to as smart filtering), followed by automatic filtering and manual analysis to identify clinically relevant variants with a final review by an expert panel for diagnosis confirmation.

Results: The smart filtering method identified clinically significant variants in three cases, validated by additional methods like Sanger sequencing and segregation analysis. This increased the overall diagnostic yield by 3%, supporting the hypothesis that smart filtering can enhance variant detection. In addition, novel variants of unknown significance were identified in 25 cases.

Conclusion: Incorporating non-coding regions using relatively simple filtering criteria in the genomic analysis process demonstrates potential to improve diagnosis rates in rare diseases, especially where conventional methods are unrevealing.

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P16.069.D Study of the carrier frequency of *SMN1*-related spinal muscular atrophy in southern India and development of a cost-effective quantitative PCR-based test for SMA carrier screening

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Background: Carrier frequency of *SMN1*-related spinal muscular atrophy is reported to be around 1 in 40 to 100 in different ethnic groups, but not well studied in the southern Indian population. This study aimed to determine carrier frequency of SMA in southern India, and to develop a cost-effective quantitative PCR-based strategy for SMA carrier screening.

Material and Methods: Carrier status data of 645 individuals (133 males and 512 females) who underwent routine SMA carrier screening through the standard MLPA method at a medical genetics centre in southern India, was collected prospectively over a period of two years (from January 2023 to December 2024). Further, qPCR was done for 60 randomly chosen individuals from the routine carrier screening cohort and for 51 parents of SMA-affected children with homozygous exon 7/8 deletion.

Results: Sixteen of 645 individuals (2.48%) tested carrier positive. qPCR results for 60 individuals from this cohort showed 100% concordance with MLPA results. Among 51 parents of SMA-affected children, 44 tested positive and 7 tested negative for heterozygous deletion carrier status through both qPCR and MLPA; these 7 presumably have 2+0 carrier status which needs further confirmation. The calculated cost per sample for qPCR-based testing was around half that for MLPA.

Conclusion: Carrier frequency of SMA in our study population was found to be around 1 in 40, similar to world-wide figures. However, being a hospital-based study, this may not truly represent carrier status of the general population. qPCR can be used as a cost-effective strategy for population-based carrier screening.

C.O.I. Disclosure:

P. Ranganath: None. **R. Balan:** None. **V.R. Sarvade:** None. **A. Dalal:** None.

P16.070.A Utilization of Twist CNV Backbone Spike-in Panel with exome for cytogenetic research

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Background: Array comparative genomic hybridization (aCGH) has been the gold standard for detection of copy number variation (CNV). NGS is becoming a standard tool for clinical lab, enabling platform consolidation into an all-in-one NGS assay to replace aCGH.

Material and Methods: We describe here a method to leverage Twist Bioscience's double-stranded DNA probes that can be individually tuned to enrich regions of interest with exceptional uniformity, reducing the overall cost of sequencing. With a proprietary algorithm we positioned probes in the intergenic and large intronic regions to create a genome-wide backbone of probes that fill the gaps between exome targets. Twist CNV Backbone Spike-in Panels were designed on common and polymorphic SNPs in multiple populations at 3 different probe densities, ranging from highest resolution to lowest at 25 kb, 50 kb, and 100 kb spacing to accommodate various requirements.

Results: We will present data that illustrates target enrichment efficiency using Twist's complete workflow through diverse samples that harbor a clinically significant chromosomal aberration. We will also demonstrate the 100% concordance of CNVs called between NGS and aCGH data. Moreover, we will show how aCGH data output could be replicated using this NGS solution.

Conclusion: The data will demonstrate how Twist's all-in-one assay lets researchers sequence more deeply and more efficiently into each sample. The combination of Twist CNV Backbone Spike-in Panels with Twist Exome Panels enables detection of genome-wide detection of CNVs or LOH, on top of SNVs and small InDels that come with Twist Exome 2.0 plus Comprehensive Exome Spike-in Panel.

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P16.071.B The unseen crisis: why genetic testing is failing Greek lung cancer patients

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Background: Lung cancer (LCa) is the leading cause of cancer-related deaths worldwide and the third leading cause in Greece.[1,2] Currently, there are 11 LCa biomarkers, including *EGFR*, *KRAS*, *ALK*, *MET*, *ROS1*, *BRAF*, *RET*, *HER2*, and *NTRK1/2/3*, that offer opportunities to expand personalised treatment strategies.[3] Unfortunately, in Greece, the identification of actionable variants is not in sync with LCa patients' access to comprehensive genetic testing as national health insurance policies cover testing for only three of them (*EGFR, KRAS, ALK*). This discrepancy leads to healthcare inequalities, ultimately increasing patient mortality rates.

Material and Methods: For 2023 and 2024, variants in the aforementioned genes were identified in-house using either a 3-gene panel (Sanger sequencing and FISH) or a 51-gene panel (Next-Generation Sequencing).

Results: Among 110 LCa patients, 40 opted for the 3-gene panel testing (group A), while 70 chose the 51-gene panel testing (group B). In group A, a clinically significant variant (CSV) was identified in 15% of the cases, whereas in group B, it was present in 48.5%. In group B, approximately 24.2% of the patients had a CSV detected in one of the three genes and a further 24.2% had the CSV identified in the other actionable genes that would have been missed if they had undergone the 3-gene panel testing.

Conclusion: Understanding the importance of expanding the existing genetic panel is essential for providing precise treatment of patients with LCa. Biomarkers are a vital part of personalised medicine, which is an imperative approach to prolonging patients' life.

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P16.072.C Diagnosis of myeloproliferative neoplasms: the pivotal importance of Sanger sequencing in the detection of *CALR* gene mutations

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Background: The discovery of *CALR* gene mutations represented a major breakthrough in the molecular diagnosis of BCR-ABL-negative myeloproliferative neoplasms (MPN). These mutations are found in approximately 80% of essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients who don't carry mutations in the *JAK2* or *MPL* genes. The objective of our study is to demonstrate the contribution of Sanger sequencing in identifying recurrent mutations of the *CALR* gene and thus in the diagnosis of MPN.

Material and Methods: We report in this study a series of 21 patients referred to our medical genetic laboratory at Mohammed VI University Hospital Center of Oujda for suspected BCR-ABL negative MPN. DNA was extracted from peripheral venous blood. After conventional PCR amplification of the exon 9 of the *CALR*

gene, the DNA fragments were purified and sequenced by the Sanger method on ABI SeqStudio.

Results: The molecular analysis revealed three distinct pathogenic mutations in the *CALR* gene in 6 patients (28.57%). All patients harbored deletion/insertion mutations; two had type 2 recurrent 5 bp insertion (c.1154_1155insTTGTC), one presented type 1 recurrent 52 bp deletion (c.1092_1143del52). The variant c.1099_1150del51 was identified in 3 patients.

Conclusion: The present study underscores the utility of Sanger sequencing-based molecular analysis for the identification of recurrent *CALR* gene mutations in myeloproliferative neoplasms, thereby enabling earlier diagnosis and improved patient management.

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P16.073.D ONTOPREC: Precision-Ontology: ONTOPREC. Implementation of ONTOlogies, taxonomies and standardization of chronic diseases in PRECision Medicine. Use of polygenic risk scores (PRS) as use cases

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The ONTOPREC project advances precision medicine by standardizing socio-health variables relevant to chronic diseases, major contributors to morbimortality and healthcare costs. It leverages ontologies, taxonomies, and standardized electronic health record (EHR) formats to integrate and analyze clinical data. The computation of polygenic risk scores (PRS) through whole genome sequencing (WGS) serves as a use case, demonstrating the benefits of structured data integration and bioinformatic analysis. ONTOPREC aligns with international standards, FHIR and OMOP, and employs natural language processing (NLP) to enhance data interoperability.Through a consensus-driven approach, ONTOPREC has standardized 20 socio-health variables, mapping each to specific ontologies, vocabularies, and synonyms to ensure FHIR and OMOP compatibility. NLP has facilitated variable extraction, mapping, and structuring. A pilot study with 200 WGS samples assessed the feasibility of integrating genetic risk scores into EHRs.ONTOPREC highlights the significance of standardization in precision medicine, providing a scalable framework for chronic disease data integration. The successful consensus on socio-health variables and ontological mapping supports harmonizing datasets for improved patient stratification and decision-making. The project also underscores NLP's role in enhancing unstructured clinical data usability and the importance of genetics in PRS prediction. Future efforts will validate the proposed standards' interoperability across healthcare systems and expand the scope to additional conditions and variables. These findings promote the adoption of standardized EHRs and genetic risk assessments in clinical practice.

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P16.074.A Assessing extraction efficiency of DNA for long read sequencing

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GenQA, Edinburgh, United Kingdom.

Background: Increasing numbers of laboratories are looking towards long read sequencing (LRS) to provide more in-depth analysis of DNA. The quality of DNA extraction is a crucial aspect of this process for successful downstream testing. However, there currently is not a way for laboratories to benchmark their extraction process with other diagnostic laboratories.

Material and Methods: GenQA developed an external quality assessment (EQA) to assess the quantity and quality of DNA extracted from blood for LRS. Laboratories were sent a 1ml blood sample from the same source, and were instructed to extract DNA using their routine protocol for LRS and return the sample to GenQA for evaluation.

Results: DNA yield was measured by ddPCR, quality by a reference laboratory using Agilent femto pulse, and volume determined by sample weight.

Sixteen laboratories participated using 8 different extraction methods. One laboratory participated twice, with/without a clean-up step.

The yield of DNA ranged from 4.5 to 29.5 µg. The GQN10kb score, which is the proportion of DNA >10 KB is scored from 0–10. The GQN10kb ranged from 4.6 to 9.6. The laboratory that participated twice had a GQN10kb score of 6.2 for extraction protocol and a score of 9.6 for extraction and clean-up protocol.

Conclusion: The quality of DNA extracted was highly variable, demonstrating the benefit of the EQA for laboratories to identify areas of improvement. The increase in GQN10kb score by the laboratory following the clean-up step highlighted that in some circumstances, it would be beneficial to include this aspect to the extraction protocol.

C.O.I. Disclosure:

F.E. Moon: None. **Z. Deans:** None.

P16.075.B Could aneurysm and atherosclerosis-associated microRNAs (miR 24-1-5p, miR 34a-5p,miR 126-5p, miR 143-5p, miR 145-5p) also be associated with coronary artery ectasia?

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Background: Coronary artery ectasia (CAE), known for localized or diffuse excessive dilatation of the coronary artery, has an unknown etiology, but it has been reported that the underlying cause may be atherosclerosis and genetic changes that may affect the arterial lumen. MicroRNAs (miRNAs) have been shown to have an effect in aneurysm diseases, and atherosclerosis. The purpose ofthis study was to investigate whether they are also associated with CAE.

Material and Methods: This cross-sectional study consisted of 25 patients with CAE and 25 subjects with normal coronaryarteries. Blood was collected, and miRNA expression was detected using the Rotor-GeneQ real-time poly-merase chain reaction cycler (Qiagen, Dusseldorf, Germany) to investigate expression levels of miR-24-1-5p,miR-34a-5p, miR-126-5p, miR-143-5p, and miR-145-5p.

Results: Demographic variables of CAE (mean age 59.5–1.7; 12 women) and controls (mean age 57.2–2.1; 16 women) were similar. miR-126-5p ($p = 0.014$) and miR-145-5p ($p = 0.003$) levels were found to be less than two fold upregulated in CAE compared with controls; miR-143-5p also showed upregulation, but it was not significant ($p = 0.078$). On the contrary, miR-24-1-5p ($p = 0.032$) levels were downregulated in CAE compared with controls. miR-34a-5p was also downregulated, but this was not considered significant ($p = 0.185$).

Conclusion: According to our study findings, miR-126-5p, miR-145-5p, and miR-24-1-5p may be associated with CAE. We think that these miRNAs could be of therapeutic significance for further studies of CAE involving abnormal angiogenesis and vascular disorders, and potentially serve as useful biomarkers.

C.O.I. Disclosure:

Z. Yalım: None. **S. Tutgun onrat:** None. **&. Dural:** None. **E. Onrat:** None.

P16.076.C Comparative analysis of whole genome sequencing (WGS) diagnostic yield for multiple variant interpretation platforms in genome diagnostics for genetic disorders

***Christophe Debuy**, Rachel Schot, Federico Ferraro, Rick van Minkelen, Adriana Iglesias Gonzalez, Frank Sleutels, Lisette Meerstein - Kessel, Stefan Barakat, Tjakko van Ham*

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Background: Despite the immense impact of exome sequencing (ES) in genetic disorders, still in only 20–50% patients with genetic disorders, the genetic cause is identified. Since 2024, ES has been gradually replaced by WGS with the potential to identify all pathogenic variants but also resulting in an abundance of variants of uncertain significance. To improve the efficiency of genetic diagnostics, it is imperative that reliable and accurate variant interpretation software is used. Here, we will determine and compare the diagnostic yields in our WGS cohort using the previously used Alissa Interpret software and the recently implemented Emedgene variant interpretation software.

Material and Methods: In a retrospective analysis, we compared both variant interpretation software platforms using the same patient cohorts ($N = 297$). This will determine whether causative variants identified using Alissa Interpret, will also be detected and prioritized by AI in Emedgene. Conversely, currently undiagnosed patients analyzed with Alissa Interpret, will be re-analyzed using Emedgene.

Results: In this retrospective analysis, a total diagnostic yield of 22.89% (26.60% including candidate causative variants) was obtained (61/297) using Alissa when WGS was performed first. In patients who had previously undergone WES analysis that yielded no genetic diagnosis, WGS provided an additional diagnostic yield of 8.73% (10.91% including candidate causative variants).

Conclusion: Insights in which analysis platform would uncover more potentially pathogenic variants in unsolved patients, will help improve the diagnostic yield in rare and genetic disorders and the efficacy of genomic medicine in clinical practice.

C.O.I. Disclosure:

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P16.077.D Streamlining genomic diagnostics: Integration of CNV data into a clinical decision support system for enhanced NGS variant classification at NEYGLH

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Background: NGS results plays a crucial role in the NHS Genomic Medicine Service by diagnosing rare genetic diseases. A key aspect of this service is variant classification, where Copy Number Variants (CNVs) help in identifying the genetic cause of a patient's phenotype. Currently, the Northeast and Yorkshire Genomics Laboratory Hub uses the in-house decision support software VarDB. However, CNV data generated by tools such as DECoN is managed separately, resulting in a fragmented and time-consuming analysis. This project aims to develop a software feature within VarDB that incorporates CNV data alongside existing variant information, streamlining analysis, enabling automated first checks, and facilitating long-term trend analysis.

Material and Methods: An AGILE Software development lifecycle consisting of 3 overarching Epics was used. Epic 1 focused on the system architecture, software design, and gathering user requirements from Clinical Scientist. Epic 2 focused on developing a platform that integrates backend database with filtering algorithms, and a front-end interface for CNV results. Epic 3 consisted of validation and testing, such as performance evaluation using both in-silico and patient data, gathering stakeholder feedback to improve the software.

Results: Design outputs, including system schematics and database models, were created to guide development. These models ensure compatibility with existing VarDB software and future extensibility for additional CNV callers. Preliminary evaluations suggest that the integration reduces analysis turnaround times and complexity.

Conclusion: Final results including validation, impact assessments, and deployment outcomes will be presented at the conference, demonstrating any significant changes in workflow efficiency and patient-focused diagnostic turnaround times.

C.O.I. Disclosure:

M. Baines: None. **J. Mahon:** None. **A. Jones:** None.

P16.078.A Overcoming diagnostic challenges: The value of multi-omics approaches for gene-pseudogene constellation

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Background: Pseudogenes complicate the short-read sequencing-based detection of copy number variants (CNV). This is due to read misalignment by gene variants that are representative of pseudogene sequence (or vice versa). Orthogonal MLPA testing is therefore recommended as an aid to confirm the presence of a CNV.

Material and Methods: We analyzed a female by exome sequencing in the scope of pre-IVF carrier screening. A putative single-exon deletion triggered MLPA. In addition, a fragment containing the exon of interest plus the two neighboring exons was analyzed by Nanopore long-read sequencing.

Results: Exome sequencing suggested a heterozygous deletion of exon 3 of the *IDS* gene, suggestive of carriership for the X-chromosome Hunter syndrome. Subsequent MLPA was consistent with this interpretation. Since the MLPA probe is indicated to be sensitive to DNA quality issues based on high GC content, we additionally performed long-read sequencing. There was no evidence for the suspected deletion, but we detected several heterozygous intronic variants. All of these represent pseudogene reference sequence, arguing for a copy-neutral pseudogene-gene conversion. One of the variants (rs470986) is known to cause misalignment of gene-derived reads to the pseudogene, explaining the initial exome sequencing observation. Another variant (rs1734484) overlaps with the ligation site of the critical MLPA probe, explaining the apparent MLPA confirmation.

Conclusion: A disease-association of the conversion is currently investigated by RNA sequencing. Our case highlights that for genes with a pseudogene, short-read sequencing + MLPA may be insufficient regarding CNVs. Instead, a multi-omics approach should be implemented.

C.O.I. Disclosure:

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P17 Bioinformatics, Machine Learning and Statistical Methods

P17.001.B Streamlining selection of variants for functional studies to reclassify variants of unknown significance

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Background: 75% of rare disease cases in the 100,000 Genomes Project remain unsolved. Functional studies allow reclassification of VUS; however, application to all VUSs is prohibited by cost and throughput limitations. Identifying variants that are most likely to benefit from functional studies will allow targeted follow-up testing.

Material and Methods: We utilise Exomiser scores, in-silico pathogenicity prediction tools and panelApp information to prioritise variants in specific genes from the unsolved 100,000 Genomes cohort.

These variants will be passed to research teams for functional analysis. Any findings which may result in a new diagnosis will be returned via the Genomics England Diagnostic Discovery pathway.

Results: For initial tool assessment, unclassified variants were compared to the outputs of previous SGE functional studies to assess variants from BAP1 (Waters et al, 2024) and DDX3X (Radford et al 2023). 4 out of 6 (66 %) returned BAP1 variants were classified as "depleting", and 2 out of 9 (22%) DDX3X variants were classified as "enriching". Tool application to the unsolved 100,000 Genomes cohort returned 1082 variants across 42 genes. Of these, 18 variants are within genes which are PanelApp green for the referral panel. Functional analysis is currently on going.

Conclusion: Our method prioritises variants that are likely to provide functionally actionable results, which can provide evidence of pathogenicity to VUS, in the long-term increasing the diagnostic yield of sequencing.This research was made possible through access to data and findings in the National Genomic Research Library via the Genomics England Research Environment.

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E. MacKenzie: None. **E. Reid:** None. **J. Ahn:** None. **M. Garner:** None.

P17.002.C Next-generation phenotyping advancing ACMG variant classification and newborn screening, demonstrated through Alagille syndrome

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Background: While next-generation phenotyping (NGP) is widely recognized for its diagnostic potential, its application to ACMG variant classification and newborn screening remains underexplored. Alagille syndrome (ALGS), an early-onset multisystem disorder with available medical therapies to prevent severe complications, serves as a compelling model to explore these applications. We demonstrated how NGP facilitates the reclassification of variants of uncertain significance (VUS) and enables newborn screening, particularly in diverse and underserved populations.

Material and Methods: Our model was trained on 121 ALGS patients from diverse ancestries, sourced from the GestaltMatcher Database, and validated against healthy controls. For newborn screening, ALGS cases were compared to healthy infants (age <1 year) from the UTKFace dataset. We encoded each image into 512 dimensional feature vectors and trained a Support Vector Machine (SVM) to classify between ALGS and healthy controls. Thresholds for ACMG PP4 criteria (supporting, moderate, strong) were established using a Bayesian framework.

Results: We established robust thresholds for PP4 criteria to facilitate VUS reclassification, enabling more confident variant interpretation. Our analysis highlighted performance differences across age groups and ancestries, underscoring the importance of diverse training data. In a simulated newborn screening scenario, our SVM model achieved a Positive Predictive Value (PPV) of 90%.

Conclusion: NGP expands beyond diagnostics to address gaps in ACMG variant classification and newborn screening, as demonstrated by its application to ALGS. This approach facilitates early intervention, reduces reliance on genetic expertise, and improves outcomes in underserved populations, highlighting its potential to advance global precision medicine.

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P17.003.D Investigating variant pathogenicity prediction by examining the performance of in-silico predictive tools using clinical data

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Whole genome sequencing (WGS) data for patients with rare disorders are being interpreted clinically by NHS laboratory scientists through the 100,000 Genomes Project and NHS Genomic Medicine Service (GMS). The resulting variant classifications can be applied to evaluate and improve in-silico tool prediction. Here we present an interpretable and quantitative framework that identifies overlapping thresholds for error minimisation (FPR and FNR) and precision maximisation (PPV and NPV) for 10 in-silico algorithms applied to a cohort of missense variants (88.3% pathogenic / 11.7% benign) clinically interpreted as part of the 100,000 Genomes Project or GMS. We introduced a threshold of 0.75 for minimum precision and 0.3 for maximum error, resulting in BayesDel and MetaRNN presenting with overlapping thresholds, with the others unable to satisfy all four metrics. BayesDel's decision-thresholds range from 0.369 to 0.379 (FPR < 0.1, FNR < 0.2, PPV, NPV>0.75) and MetaRNN's from 0.291 to 0.317 (FPR < 0.1, FNR < 0.3, PPV, NPV>0.75). PR-AUC comparisons (ranking BayesDel first with a score of 80.6, followed by ClinPred (75.6) and AlphaMissense (71)) fail to reflect the observed high FNR and low NPV of ClinPred and AlphaMissense, highlighting the benefit of this framework. When investigating the impact of protein structure and organisation we noted that BayesDel outperformed in alpha helix domains (FPR, FNR < 0.3, PPV, NPV>0.7), and ClinPred in beta sheet domains (FPR, FNR < 0.25, PPV, NPV>0.68). Overall, this framework enables users to base their decision threshold selection on their desired key metric, allowing for trade-off analyses across metrics and models at both the patient-level and specific subsets of domain-associated variants.

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P17.004.A Phenome-wide health associations of multi-allelic copy number variants in UK Biobank

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Background: The contribution of multi-allelic CNVs (mCNVs) to disease risk has not been widely studied, as they are difficult to characterise at large-scale genome-wide, and are often not strongly associated with flanking SNVs, limiting imputation approaches. Improved understanding of the role of mCNVs in disease risk could lead to novel insights into the pathobiology of disease.

Material and Methods: We used ClinCNV to robustly type 69 mCNVs from UK Biobank whole exome sequences in discovery (n = 152,853) and replication sets (n = 269,317). Discovery and replication PheWAS of these variants used DeepPheWAS, which interrogates clinically-curated composite phenotypes by integrating self-report, primary and secondary health care data. Separate PheWASs were conducted for unrelated individuals of African, European and South Asian ancestries. The false discovery rate (FDR) threshold for significant discovery associations was 0.01 for European ancestries and 0.05 for the smaller African (n = 1,943) and South Asian (n = 3,085) ancestries. Associations replicated when the direction of effect was consistent in discovery and replication sets and the replication p-value for association was less than 0.05.

Results: 174 mCNV-phenotype associations were detected from 26 mCNVs, of which 114 associations replicated. Four of eight potentially novel mCNV-phenotype signals were independent of neighbouring associated SNPs, including *SULT1A1/SULT1A2* with eGFR in Europeans (meta-analysed $p = 1.05 \times 10^{-9}$, beta = 0.016 [0.011; 0.021]), and *GOLPH3* with bundle branch block in South Asians (meta-analysed $p = 3.35 \times 10^{-6}$, OR = 2.25 [1.16, 4.37]).

Conclusion: We show that accurate typing and large sample sizes can reveal genetic associations of mCNVs, and our work provides several novel likely causative variants contributing to clinically-important traits.

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P17.006.C Imputation and phasing methods impact polygenic scoring with low-coverage sequencing data across 27 diseases and traits

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Background: Low-coverage whole-genome sequencing (lcWGS) is increasingly used in research to produce genome-wide genotypes. LcWGS offers a cost-effective alternative for array genotyping for polygenic risk scores (PRS) and is less prone to variant ascertainment bias. However, its performance for PRS calculation remains understudied. Here we compare imputation and phasing methods for lcWGS and the impact of coverage on PRS precision.

Material and Methods: We assessed the utility of imputed lcWGS data in PRS estimation, using 30x WGS data for 24 Finnish individuals with six downsampled low coverages (0.1x-2x) and a population-specific imputation panel. We compared three imputation and phasing approaches (Beagle4, Beagle5 and GLIMPSE2) across 27 disease and trait PRSs covering a great number of non-communicable diseases and their risk factors.

Results: Imputation and PRS calculation concordance compared to high-coverage WGS increased with lcWGS coverage in all approaches. LcWGS imputation with GLIMPSE2 outperformed the other two approaches, both in imputation and PRS concordance ($p < 1\times$;10-21 and $p < 1e\text{-}8$), with 0.5x reaching imputation and PRS concordance of $r2 > 0.90$. WithGLIMPSE2, PRS risk category reclassification was also minimal (all ≤10%) starting from 0.5x. Differences in PRS concordance between disease and trait PRSs were detected, with PRSs containing single high-impact variants showing poorer concordance.

Conclusion: Processing lcWGS data with dedicated imputation and phasing methods, such as GLIMPSE2, is important for optimal PRS calculation. With such approaches, coverage as low as 0.5x can achieve PRS performance comparable to genotyping arrays, making lcWGS a feasible option for PRS studies.

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P17.007.D Detection of short tandem repeat expansions from exome sequencing data in patients with neurological conditions

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Background: Diagnosing neuromuscular and neurodegenerative disorders presents a significant global healthcare challenge due to their genetic heterogeneity and the presence of elusive genetic variant types such as short tandem repeat (STR) expansions. These factors often contribute the elongation of an already complex diagnostic process of genetic diagnostics.

Material and Methods: We evaluated 714 patients referred for genetic diagnostics to our institution between 2013 and 2025, who presented with neuromuscular or neurodegenerative disorders and had negative exome or genome sequencing results. Based on symptoms compatible with tandem repeat disorders, 76 were selected for bioinformatic STR profiling using Expansion Hunter.

Results: In selected patients we identified 17 clinically relevant STR expansions. Pathogenic expansions were found in HTT (six patients), ATXN8OS (four patients), AR and DMPK (two patients each), and CNBP, FMR1, and FXN (one patient each).

Conclusion: Employing STR profiling we achieved a diagnostic yield of 22.4% in the selected cohort of patients and 2.4% in the overall group of patients with neuromuscular and neurodegenerative disorders. Bioinformatic characterization of STRs from exome and genome sequencing data proved to be a reliable method, with all variants confirmed by gold standard triplet repeat-primed PCR.

C.O.I. Disclosure:

G. Bergant: None. **B. Peterlin:** None.

P17.008.A Proteome wide prediction of mode of inheritance and molecular mechanism underlying genetic diseases using structural interactomic

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Background: Genetic diseases are classified by their modes of inheritance (MOI) and molecular mechanisms. Autosomal dominant (AD) disorders arise from loss-of-function, gain-of-function, or dominant-negative variants, while autosomal recessive (AR) diseases are linked to loss-of-function variants. This study introduces a graph-of-graphs approach leveraging protein-protein interaction (PPI) networks and high-resolution protein structures to predict MOI and classify functional effects of AD proteins. Using graph neural networks (GNNs) integrated with structural interactomics, the method enables proteome-wide predictions, enhancing understanding of genetic disease mechanisms.

Material and Methods: Data on MOI and molecular mechanisms were collected for 4,737 proteins, categorized as AR (53%), AD (30%), and both (ADAR, 17%), with functional effect data for 1,276 AD proteins. A comprehensive PPI network with 17,248 nodes and 375,494 edges was constructed using STRINGdb, BioGRID, and other resources, enriched with protein structure data from AlphaFold and annotated with 78 protein features and 73 residue features.

Results: Models were developed using several GNN architectures, including graph attention, convolution, and isomorphism network (GAT, GCN, and GIN, respectively) and evaluated via precision, recall, and F1 scores. The GAT model outperformed previous state-of-the-art models for MOI prediction ($F1 = 0.74$), and the GCN was good at functional effect classification ($F1 = 0.61$). Applying the models to the human proteome, 16,184 autosomal proteins were classified, revealing pathway enrichments linked to cancer for AD and mitochondrial or neurodevelopmental roles for AR proteins.

Conclusion: This framework bridges network-level and structural insights, offering interpretable tools for genetic research. Future work will incorporate tissue-specific data.

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A. Saadat: None. **J. Fellay:** None.

P17.009.B PGS-Optimizer: A federated and modular framework for enhancing polygenic risk Score computation in diverse populations

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Background: Polygenic scores (PGS) are widely applied in genetic risk prediction, yet their accuracy is constrained by the dominance of genome-wide association studies (GWAS) in European populations. This limitation reduces the clinical utility of PGS in diverse

populations. Additionally, developing new PGS models is a complex task that requires significant effort, including data integration, algorithm selection, and rigorous evaluation. PGS-Optimizer is introduced to address these challenges through a federated and modular computational framework.

Material and Methods: PGS-Optimizer integrates cutting-edge PGS algorithms—PRS-CSx, PRScise2, Ldpred2, Lassosum2, and PLINK—within a federated learning infrastructure that facilitates secure, cross-institutional collaborations while preserving data privacy. The platform includes preprocessing modules for genotype quality control and imputation, ensuring consistency across datasets. Performance evaluation was conducted using synthetic datasets generated via HAPNEST, representing six distinct populations with varying sample sizes to assess the robustness and scalability of the framework.

Results: The platform demonstrated significant improvements in PGS accuracy and reproducibility across diverse population datasets. The federated learning approach provided secure model development across multiple institutions, while the modular architecture enabled efficient workflow customization.

Conclusion: PGS-Optimizer offers a scalable, reproducible, and privacy-preserving solution for advancing PGS research across diverse populations. Our tool is a continuously evolving platform, with regular updates planned to enhance functionality and expand population coverage. Future directions include the integration of machine learning-based risk models, expanded compatibility with emerging genomic datasets, and improved reporting capabilities.

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P17.010.C A novel computational framework for differential and QTL transcript usage in single-cell data

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Background: Alternative transcript usage is a key regulator of gene expression, shaped by mechanisms such as alternative splicing and polyadenylation. Human individuals differ in their transcript usage across tissues and traits. Such differences are partially controlled by genetics and ultimately contribute to phenotypic variation. However, most studies have focused on bulk tissue level, masking cell type-specific regulation of transcript usage. The advent of large single-cell RNA-seq cohorts provides an opportunity to resolve these differences at single-cell resolution. However, two major challenges remain: transcript quantification in end-biased scRNA-seq data, and the lack of single-cell differential transcript usage methods suited for complex experimental designs.

Material and Methods: To address these limitations, we developed a novel computational framework for differential and QTL transcript usage analysis in single-cell data. Our approach tackles the coverage bias of scRNA-seq data by performing equivalence class quantification and models transcript usage differences between individuals using a non-parametric multivariate test.

Results: We applied our framework to a scRNA-seq dataset of immune blood cells from 80 European and 80 African individuals in baseline and under viral infection: influenza and covid. Both infections induced widespread transcript remodeling across cell types, particularly in monocytes, and affected immune response upregulated genes. Population differences in transcript usage were enriched for genetic variants associated with splicing regulation and were independent of gene expression changes.

Conclusion: Overall, we introduce a computational strategy to study transcript usage variation across multiple phenotypes in single-cell data, expanding the understanding of splicing and polyadenylation diversity across individuals in response to viral infection.

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R. Chazarra-Gil: None. **I. Mestres-Pascual:** None. **M. Melé:** None.

P17.011.D aiDIVA: Classification and prioritisation of causal variants in rare disease patients using Large Language Models

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Background: Genome sequencing is a widely used diagnostic tool for detection of genetic disease variants. However, variant interpretation and identification of causal variants in patients with rare diseases (RD) is challenging and time-consuming and requires trained experts. Hence, there is a need for AI-driven clinical decision support systems that assist clinical experts in identifying causal variants or, in case of large-scale re-analysis of unsolved cases, automate the process.

Material and Methods: We introduce aiDIVA, a tool featuring multiple machine learning models trained on genomic and phenotypic data, to identify the causal variant of an RD patient. First, aiDIVA uses features based on genomic and clinical annotations to generate a pathogenicity score for each variant using 1) a random forest AI, and b) an evidence-based model. aiDIVA combines these models with clinical metadata (e.g., phenotype as HPO terms) to rank the most likely causal variants. Second, aiDIVA uses a similarity approach comparing disease phenotypes associated with a certain gene to the clinical information of the patient. Third, aiDIVA uses large language models (ChatGPT, LLaMA) to rank the top-10 candidates. Finally, a meta-AI model combines all scores to generate the final ranking.

Results: In a benchmark analysis with more than 3000 diagnostically solved RD patients of the University Clinics Tübingen, aiDIVA reported the causal variant in 97% among the top three candidates. Furthermore, aiDIVA provides interpretable explanations for each candidate.

Conclusion: aiDIVA has the potential to speed-up diagnostics by automatically indicating the causal gene in 97% of the solvable RD cases.

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P17.012.A The Ensembl Variant Effect Predictor (VEP) and human pangenome assemblies: as featured on the new Ensembl beta website

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EMBL-EBI, Hinxton, United Kingdom.

Background: The Human Pangenome Reference Consortium (HPRC) aims to create a genome reference which better represents human genetic diversity by combining high quality haplotype assemblies from hundreds of individuals of different ancestries. Ensembl is a member of the HPRC and to further this ambition, the new Ensembl beta website (www.beta.ensembl.org) provides enhanced support for multiple, more diverse human genome assemblies. In addition to human GRCh37 and GRCh38, Ensembl beta supports the T2T-CHM13 assembly alongside 94 human haplotype assemblies produced by the HPRC, variant views and the capability to run a core version of Ensembl VEP is enabled for all these assemblies.

Material and Methods: Individual gene annotations were constructed using a scalable and accurate approach, centred on the use of local alignments of genes and gene clusters between the GRCh38 reference and target genomes. We lifted over variants from dbSNP156 to display on the new assemblies.

Results: Gene and variant views for the human pangenome assemblies are available on the new website as summary tracks on the Genome Browser and in greater detail in our Entity Viewer. Ensembl VEP enables the annotation and prioritisation of genomic variants on the pangenome assemblies.

Conclusion: Ensembl now enables more detailed visualisation and annotation of variants discovered against new, more diverse human haplotype assemblies, supporting the global effort in human genomics. These efforts enable identification of population-specific genetic variants that affect disease risk, treatment efficacy, and drug responses, which are crucial for realising the benefits of precision medicine.

C.O.I. Disclosure:

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P17.013.B Re-identifying Genomes by Linking Polygenic Scores to Phenotypic Traits

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Background: Access to an individual's genome can reveal sensitive information, such as their predisposition to certain diseases. To enable scientific research while protecting participants' privacy, researchers anonymize genomes by removing identifying information (e.g., names or zip codes). However, studies suggest that it may still be possible to re-identify a genome, even if fully anonymized, by inferring genetically predicted phenotypes and comparing them to the traits of known potential genome owners.

Material and Methods: Here, leveraging recent advances in GWAS, we investigate the feasibility of such re-identification. We introduce an approach that compares multiple traits of a target individual with the Polygenic Scores (PGSs) derived from a query genome to infer the probability that the genome belongs to the individual. Briefly, our approach learns the underlying residual distributions in a training dataset and uses a likelihood-ratio test (LRT) to decide whether a PGS profile and a set of traits belong to the same individual. We benchmarked our approach by extensive simulations of re-identification attempts using genomes and phenotypes from the UK Biobank.

Results: Re-identification accuracy increased with the number of traits used. Using 40 traits, for more than half of tested individuals, our LRT would guess the correct match with an error rate of 0.17%. Finally, we assessed how our approach could be leveraged to infer other unmeasured traits of the target individual and compared it to phenotype-based inference strategies.

Conclusion: We show that advances in phenotype inference raise privacy concerns, highlighting the need for careful handling of genomic data.

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T. Cavinato: None. **R. Hofmeister:** None. **A. Malaspinas:** None. **Z. Kutalik:** None.

P17.014.C Clinical Evaluation of an AI System for Streamlined Variant Interpretation in Genetic Testing

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Background: The growing use of whole exome/genome sequencing for diagnosing hereditary diseases has increased the interpretive workload for clinical laboratories. Efficient methods are needed to identify pathogenic variants and maximize diagnostic yield without overwhelming resources.

Material and Methods: We developed DiagAI, an AI-powered system trained on 2.5 million ClinVar variants to predict ACMG pathogenicity classes. DiagAI ranks variants, proposes diagnostic shortlists, and identifies probands likely to receive molecular diagnoses. It integrates molecular features, inheritance patterns, and phenotypic data when available. We retrospectively analyzed 966 exomes from a nephrology cohort, including 196 with causal variants and 770 undiagnosed cases.

Results: DiagAI identified 94.9% of causal variants in diagnostic exomes with HPO terms, compared to 90.8% without, with median shortlist sizes of 12 and 9 variants, respectively. It achieved a sensitivity of 57.1% and a specificity of 92.6% in tagging exomes likely to contain a diagnostic variant. With HPO terms, 74% of top-ranked (top 1) variants were diagnostic, versus 37% without, and DiagAI outperformed Exomiser and AIMARRVEL in this setting.

Conclusion: DiagAI generates accurate shortlists of variants that streamline the variant interpretation process. It provides a scalable solution for managing growing diagnostic test volumes without compromising quality.

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J. Ruzicka: A. Employment (full or part-time); Significant; SeqOne Genomics. **J. Ravel:** A. Employment (full or part-time); Significant; SeqOne Genomics. **J. Audoux:** A. Employment (full or part-time); Significant; SeqOne Genomics. **A. Boulat:** A. Employment (full or part-time); Significant; SeqOne Genomics. **J. Thevenon:** None. **K. Yauy:** None. **L. Raymond:** A. Employment (full or part-time); Significant; Eurofins Biomnis. **M. Dancer:** A. Employment (full or part-time); Significant; Eurofins Biomnis. **Y. Lombardi:** None. **N. Philippe:** A. Employment (full or part-time); Significant; SeqOne Genomics. **M.G. Blum:** A. Employment (full or part-time); Modest; SeqOne Genomics. **N. Duforet:** A. Employment (full or part-time); Significant; SeqOne Genomics. **L. Mesnard:** None.

P17.015.D PRC2-related epigenetic age acceleration in acute myeloid leukemia with *DNMT3A* and *IDH2* mutations

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Background: Age-associated DNA methylation at specific CpG sites has been developed as a biomarker that estimates the epigenetic age (EA) across human tissues. EA acceleration (EAA) is widely observed in patient cohorts, including those with hematological malignancies. Current causation analysis and biological interpretation of disease-dependent EAA remain less characterized.

Material and Methods: By harnessing causality-enriched epigenetic clocks, we determined the causal EAA for all statistically analyzable mutated genes in acute myeloid leukemia (AML) patients from TCGA. We identified two mutated DNA methylation (DNAm) associated genes (DNMT3A and IDH2) that were correlated with different damaging and protective DNAm changes during AML pathogenesis, respectively. To study the potential molecular mechanism driving EAA in AML, we profiled the DNAm status at polycomb repressive complex 2 targets (PRC2m), where has been reported to display a preferential hypomethylation manner in the DNMT3A mutated individual with clonal hematopoiesis.

Results: PRC2m from AML patients with mutated DNMT3A and IDH2 displayed distinct methylation alterations compared to other AML patients, suggesting their potential role in dysregulating the PRC2 binding landscape in the genome and the deposition of histone marks. Subsequent calculations exhibited that PRC2m was correlated with EA rather than chronological age in AML patients.

Conclusion: These findings indicate the first evidence for the biological implications of the gain or loss of methylation at PRC2 targets in mediating causal EAA in diseases. This study sheds light on the intricate connection and potential causality between genome-wide methylation alteration at PRC2 targets and the pathogenesis of AML with specific mutations.

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Y. Lei: None. **Z. Yan:** None. **S. Gu:** None.

P17.016.A Genome-wide canonical correlation analysis reveals maximally heritable disease patterns

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Background: Methods for deconstructing biobank-scale phenotype structures have relied on modelling high-dimensional phenotype data without utilizing genetic information. Here, we introduce genome-wide canonical correlation analysis (GCA), a genetically informed method for dissecting patterns from biobank observations.

Material and Methods: GCA jointly models phenotype and genetic data and outputs the most heritable phenotype patterns along with their unique genetic risk scores in the forms of phenotype and genetic latent variables. We modelled 128 heritable phecodes across 16 disease categories with 638,587 common genetic variants in a discovery set of 274,770 unrelated UK Biobank participants of White British ancestry. Latent variables and coefficients obtained from discovery set were projected in validation set (n = 68,693) for evaluation of heritability, phenotype and genetic associations.

Results: GCA retrieved 54 heritable disease patterns ($h^2 > 0$, $p < 4 \times 10^{-4}$), among which, 17 exhibited multimorbid profiles in the validation set. Disease patterns were phenotypically and genetically heterogeneous. Decoding multimorbid profiles led to biologically relevant findings. The first and most heritable disease pattern was genetically associated with altered lifespan and healthspan while the third most heritable disease pattern described opposing risks between cancer and autoimmunity. Allelic coefficients obtained from GCA also support molecular interpretation. Diverse hallmarks of aging, including telomere length, were genetically associated with the first disease pattern whiles established and novel tumor immunotherapeutic targets, including PD-1 and PTPN2, were prioritized by the third disease pattern.

Conclusion: These findings validate GCA as a genetically informed method for dissecting biologically significant patterns from biobank-scale data.

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P17.017.B A heritability-optimized method for functional prioritization of rare coding variants in complex traits

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Background: Population sequencing studies are uncovering millions of uncharacterized rare (MAF < 1%) coding variants (RV). Predicting which RVs contribute to complex disease heritability remains an ongoing challenge.

Material and Methods: We propose RovHer (Rare variant heritability-optimized scores), a machine learning approach that integrates 45 variant-level and 30 gene-level features. RovHer employs exome-wide association statistics to generate unbiased, disease-agnostic scores that reflects the probability of functional impact for each RV. Additionally, we use a novel heritability-based benchmarking approach to assess how well the RVs prioritized by these scores explain the observed variability in the trait.

Results: Analysing 21 complex traits in up to 357,086 Caucasians in the UK Biobank, the top 1% (m = 13,410) of RVs prioritized by RovHer explained an average of 16.1% of RV heritability, representing a 10-fold increase over the 7 alternative methods tested (average 1.5%; range 1.2%–2.3%). We evaluate RovHer for clinical utility, particularly in identifying carriers of missense ultra-rare variants (MAF < 0.01%) at highest risk in 20 monogenic disease-gene pairings. Notably, the top 5% of carriers (n = 143) prioritized by RovHer had significantly elevated LDL-cholesterol levels of 6.8 mmol/L (P = 1.1e-150) and 3.8 higher odds of coronary artery disease (P = 4.0e-9), numerically exceeding the 6.6 mmol/L and 3.3-fold risk (n = 23) associated with loss-of-function variants respectively.

Conclusion: RovHer leverages RV association statistics to produce unbiased scores that consistently captures the smallest proportion of likely functional variants that maximally enriches for exome-wide heritability. Our tool exemplifies the potential of prioritized missense RVs in refining population-level risk assessments.

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P17.018.C Boost statistical power to detect variants in small case cohorts leveraging gnomAD genome datasets

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Background: Detecting genetic variants associated with diseases in small cohorts (e.g. < 1000 samples because of a disease's rarity) is challenging due to limited statistical power using GWAS. Expanding the control group size is a promising approach to overcome this limitation. Utilizing aggregated variant data from gnomAD genome datasets offers a scalable solution to enhance statistical power, even with constrained case cohort sizes.

Material and Methods: This workflow integrates allele count and number data from gnomAD, allowing the selection of control datasets by ethnicity to match the case cohort optimally. Analyses can be performed at gene level or within specified genomic regions. Raw vcf files are intersected, annotated and filtered using customizable thresholds for metrics like CADD or SpliceAI and allele frequency. Allele counts are summed and divided by the average allele number. A Fisher's exact test compares case and reference cohorts, with p-values adjusted via the Benjamini-Hochberg method.

Results: The workflow substantially increases statistical power in small case cohorts, enabling the identification of significant associations with disease-causing genes. Preliminary analyses suggest it is feasible to identify unrecognized, significantly correlated genes in rare disease cohorts of individuals screened free of known disease-causing mutations, with cohort sizes as small as 200 subjects.

Conclusion: This approach leverages gnomAD datasets to bolster statistical power in studies with small case cohorts. It enables the detection of genes or regions with increased variant burden, addressing the lack of individual-level data. The workflow provides a robust, adaptable pipeline for genetic association studies.

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J. Frank: None. **M. Schlesner:** None.

P17.019.D Nallo: A comprehensive Nextflow pipeline for long-read rare disease analysis

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Background: With the latest advancements in throughput and quality, long-read sequencing (LRS) has the potential to replace existing short-read technologies as the gold standard for human genome analysis, in clinical diagnostics as well as in research. However, one major hurdle for clinical implementation is a lack of comprehensive bioinformatics pipelines that take the analysis all the way from raw data analysis to ranking and prioritisation of variants.

Material and Methods: Here we introduce Nallo, a Nextflow pipeline designed for analysis of data from both from PacBio and ONT. Nallo is easy to set up and runs on all common compute environments. Although developed for rare disease analysis, Nallo can also be used for population sequencing and other applications. The code and infrastructure adhere to the nf-core framework, which facilitates collaborative development across teams and borders[1].

Results: Nallo has been used to analyse over 100 research cases, and is currently being adopted by Swedish hospitals for the analysis of LRS data from patients affected by rare diseases. Nallo reports variants (SNVs, SVs, CNVs, TRs), which are then annotated, ranked and filtered. It also includes tools for genome assembly, methylation analysis and phasing. Nallo is openly available: https://github.com/genomic-medicine-sweden/nallo

Conclusion: Nallo provides a common analysis workflow that supports LRS research projects and translates into clinical practice. At present it is mainly used within Sweden, but we invite everyone to join the open source development and create a comprehensive LRS pipeline for the future together.

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P17.020.A HIRUscan: a framework for prioritising germline variants through medical reports and genomic data

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Background: Genomic testing for rare diseases often remains slow, complex, and resource intensive. Over the past decade, advances in human genetics have significantly improved our ability to associate genetic variants to rare diseases. However, despite these powerful technologies, most rare disease patients still fail to receive a definitive diagnosis. Consequently, unsolved cases accumulate over time across hospitals and diagnostic

laboratories, creating a significant bottleneck in rare disease diagnosis.

Material and Methods: : HIRUscan is an advanced platform designed for variant interpretation and prioritisation in diagnostic and research genetic testing. Our deep learning AI models can handle texts in English and Spanish to extract phenotypic abnormalities from medical reports. These phenotypes are encoded hierarchically, according to the Human Phenotype Ontology (HPO) terms. By combining phenotype data with genomic information, HIRUscan classifies and prioritises genomic variants using an in-house pathogenicity prediction algorithm, in conjunction with advanced ACMG/ClinGen variant classification to assess variant significance. The platform generates a ranked list of candidate variants potentially linked to the patient's clinical features, facilitating streamlined and precise identification of disease-associated variants.

Results: Preliminary results indicate that HIRUscan achieves a positive predictive value of 93% (CI 91%–94.8%) across more than 1,200 well-phenotyped and clinically characterised families.

Conclusion: HIRUscan is a software solution for fast and accurate automated variant interpretation. It presents a unique opportunity to reanalyse unresolved rare disease cases by integrating comprehensive genomic data with detailed medical reports and significantly improve diagnostic outcomes.

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P17.021.B Explainable AI for pathogenicity prediction of genetic variants in inherited heart disease

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Background: Heart disease is a leading global cause of death. Inherited heart conditions, are familial heart disorders affecting about 1 in 250 people worldwide and can impact individuals of all ages [1]. AI algorithms have shown promise in predicting the pathogenicity of genetic variants, yet traditional models often lack explainability, limiting clinician trust. Explainable AI (XAI) improves transparency, supporting better clinical decision-making. This study evaluates an XAI algorithm for predicting pathogenicity in variants linked to inherited heart disease to improve classification accuracy and build trust in AI for enhanced diagnostic and personalized care outcomes.

Material and Methods: The Random Forest (RF) model [2] was tested to predict pathogenicity of variants located in 368 genes relevant to inherited heart diseases. The model was trained on ClinVar [3] data released in November 2023 and evaluated using three datasets from ClinVar released a year later (November 2024), ensuring it was tested on unseen data: (A) 354 novel variants (177 pathogenic, 177 benign), (B) 694 conflicting variants (350 pathogenic, 344 benign), and (C) 66 reclassified VUS (28 pathogenic, 38 benign).

Results: The RF model demonstrated high accuracy: 99.44% for novel variants, 99% for conflicting variants, and 95% for reclassified VUS. The ability of the model to provide clear explanations for these predictions was a key strength, improving trust in its outcomes.

Conclusion: The RF model showed strong performance and transparent predictions, supporting clinical decision-making. Further validation and refinement of explainability are needed to enhance clinical utility and ensure reliable real-world application.

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P17.022.C Performing validity checks to support data integration for federated gnomAD releases

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Background: The Genome Aggregation Database (gnomAD) provides a reference dataset of genomic data that aids users in interpreting genetic variation. To further increase the size and diversity of this dataset, future releases will utilize a novel federated approach by incorporating aggregate frequencies from various groups otherwise restricted from sharing individual-level data, particularly sources for underrepresented groups with stricter data access policies. Ensuring the consistency and completeness of data from various sources will be essential to mitigate potential issues and delays during data integration and the production of a federated gnomAD release.

Material and Methods: We have produced a pipeline to perform validity checks on federated data. This pipeline minimizes complications at the initial stages of data integration by flagging irregularities, which includes identifying annotations with a high percent missingness, ensuring metrics align with expected patterns for chrX and chrY variants, and verifying that subset metrics aggregate to expected totals.

Results: This pipeline was tested using the gnomAD dataset and integrates previous assessments designed to prevent discrepancies in releases. The validity check pipeline is flexible in accommodating varied annotation names and formats. It outputs a report summarizing potential issues with data integrity to be resolved before proceeding with the quality control pipeline on variant data.

Conclusion: Producing a federated dataset presents new challenges requiring innovative methods. Incorporating validity checks early in the production process will help to accelerate data harmonization as we work toward producing a federated gnomAD release with high-quality allele frequency information from diverse sources of genetic data.

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P17.024.A OrthoClust: a method for improved gene clustering using single cell CRISPR screening data

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Background: Understanding complex biological systems demands the integration of multi-dimensional omics information. Single-cell-resolved CRISPR screens have become a promising tool for systematically mapping gene function in a cell. In such an experiment, many genes are perturbed in a pool of cultured cells, always one specific cell contains one gene perturbation, and the perturbation barcode can be linked to that cell's transcriptome. While this technology has high potential, better computational tools are needed to optimize functional data extraction.

Material and Methods: In this study, we propose a new computational method that integrates the two orthogonal dimensions recorded in a typical single-cell perturbation experiment, enabling more precise characterization of functional annotation. Using the first genome-scale single-cell gene perturbation dataset published by Replogle and colleagues [1], we created two types of networks: an expression profile network, derived from gene expression vectors, and a perturbation profile network, derived from gene perturbation vectors. We then combined these networks into a single integrated network, leveraging their complementary transcriptional and functional signals.

Results: The integrated network, validated against CORUM, GO:BP, and PATHWAY annotations, showed superior precision-recall metrics and better identification of biologically significant complexes [2]. Using a Precision-Recall AUC threshold of 0.3, we found that 2 complexes surpassed it in the expression profile network, 24 in the perturbation profile network, and 38 in the integrated network, demonstrating a performance gain.

Conclusion: Our findings highlight the advantages of integrating transcriptional and perturbation profile data in single-cell CRISPR screens to improve functional insight extraction.

C.O.I. Disclosure:

T. Demirtas: None. **M. Billmann:** None.

P17.025.B Genomic analysis of early-onset Parkinson's disease using long-read sequencing and optical genome mapping

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Background: While patients with Early-onset Parkinson's disease (EOPD, disease onset <50 years) often have a family history of PD, the occurrence of PD in many patients remains sporadic. The present study presents a new pipeline for structural variant (SV) detection to elucidate the contribution of pathogenic SVs in the disease etiology of EOPD by leveraging both Oxford Nanopore long-read sequencing (ONT) and Bionano optical genome mapping (OGM).

Material and Methods: ONT was performed for a discovery cohort of $N = 50$ unsolved EOPD patients. For a subset of $N = 14$, OGM was additionally conducted. A subsequent analysis pipeline based on AnnotSV, SVAFotate, ANNOVAR, and needLR was employed to detect, annotate, and filter for rare (population allele frequency ≤ 1%) pathogenic SVs and single nucleotide variants (SNVs) in known movement disorder genes or newly evaluated candidate genes. Variants (>500 bp) identified by ONT and OGM were compared using a range of ±8 kb based on the mean 95% CI of the start/end position of all OGM calls.

Results: Heterozygous deletions in *DNMT1* (123 kb), *DLG2* (398 kb), *CHMP1A* (167 bp), and *ZNF718* (46 kb) were detected. Heterozygous nonsynonymous SNVs were detected in *ITPR1*, *CHCHD2*, *PDE10A*, and *PSMF1*. About 58.6% of OGM deletions and 49% of insertions overlapped with ONT.

Conclusion: The established pipeline identified potential pathogenic variants affecting the disease etiology of EOPD. While both methods can detect large SVs, the presented comparison illustrates different aptitudes regarding insertion detection and highlights their complementary applicability. Novel variants in *PDE10A*, *PSMF1*, *DLG2*, *CHMP1A*, and *ZNF718* warrant replication in other PD cohorts.

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P17.026.C Evaluation of the efficacy of neural networks and regression analysis in predicting osteoporosis using clinical and genetic data

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Background: High hopes are placed on advanced developments in neural network training that aim to predict osteoporosis at an early stage, including the use of large amounts of genetic data. We conducted a comparative evaluation of neural network and regression analysis techniques to develop predictive risk models for osteoporosis, based on clinical factors and DNA markers of osteoporosis.

Material and Methods: The models were trained using genotype data from 152 DNA polymorphic variants and clinical parameters of 701 women and 501 men from Russia. The input parameters included anthropometric measurements, data on gender, bone mineral density (BMD) (Hologic), as well as genotyping results for candidate gene loci and replication results from a genome-wide association study (with the selection of the significant markers). Verification and testing were carried out in the Google Colab environment on the TensorFlow open source platform, as well as on the CatBoost. The Keras API was used as a high-level application programming interface for TensorFlow.

Results: The highest efficiency in neural network learning was achieved by the low BMD prediction model (AUC = 0.81), which included variants of the OPG gene (rs2073618, rs2073617, rs7844539, rs3102735, rs3134069) and variants of microRNA binding sites (rs1031820, rs6854081, rs2910164, rs10793442, rs1054204, rs11540149). The clinical and genetic model for predicting fractures in men had the highest level of prognostic

significance and included low BMD and the rs1712 variant (AUC = 0.905).

Conclusion: For the first time, high efficiency of neural network learning technology has been demonstrated for predicting osteoporosis based on risky single-nucleotide variants.

C.O.I. Disclosure:

B.I. Yalaev: None. **R. Khusainova:** None.

P17.027.D Panthera: A Transformer-Based Tool for Identifying Splicing Defects and Resolving Rare Undiagnosed Genetic Diseases

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Background: Splicing defects contribute to genetic diseases, but their detection remains challenging due to complex multi-variant interactions, often unique to population-specific backgrounds. Many pathogenic splice variants remain undiagnosed, highlighting the need for advanced computational tools in clinical genetics.

Materials and Methods: We developed **Panthera**, an algorithmic toolset leveraging a **stacked transformer encoder model** trained on CHM13 and JG3.0.0 reference genomes. Panthera predicts splicing defects across **64 distinct pangenomic backgrounds** spanning five human superpopulations, enabling detection of complex **splice haplotypes** often missed by conventional analyses.

Results: Panthera accurately identified **known pathogenic splice haplotypes** in **CFTR** and **FAS** and uncovered **three novel splice-altering variants** in **PCCB** and **MLH1**, functionally validated using minigene assays. It was instrumental to diagnose novel deep-intronic causative mutations in the Citrin gene in a Japanese pool of patients. Its ability to detect splicing disruptions from both de novo mutations and ancestral genetic backgrounds demonstrates its potential for resolving undiagnosed genetic disorders.

Conclusion: Panthera is a **clinically applicable, population-aware tool** designed to improve genetic diagnosis by identifying splicing defects that might otherwise go unnoticed. We invite **clinicians, geneticists, and researchers** to collaborate and apply Panthera in clinical cases to help diagnose **previously unexplained genetic diseases**. Our lab welcomes partnerships to further explore novel disease-associated splicing variants and improve patient outcomes.

C.O.I. Disclosure:

T. Tabaglio: None. **W. Cher:** None. **J. Ow:** None. **K.D. Wee:** None. **E. Guccione:** None.

P17.028.A A fast, sensitive, and versatile tool for annotation and differential feature detection at single cell level

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Background: RNA diversity and functionality intricately underpin the historical, present, and future states of cells and tissues. RNA sequencing serves as a transformative tool, enabling the comprehensive quantification of mRNA species and providing profound insights into underlying molecular processes. Advances in single-cell technologies, such as scRNA-seq and scATAC-seq, have revolutionized the study of transcriptional heterogeneity and chromatin accessibility, providing cell-type-specific resolution in complex tissues. However, challenges such as data sparsity and subjective annotation processes hinder the accuracy of differential feature detection and cell-type annotation.

Material and Methods: To address these limitations, we present OTMODE, a Python-based framework leveraging optimal transport theory to improve differential feature identification and annotation accuracy in single-cell multi-omics data.

Results: In our benchmarking analyses, OTMODE consistently outperformed other state-of-the-art methodologies, such as DESeq2[1], MAST[2], and SnapATAC2[3] and demonstrated robust performance across diverse simulated datasets, including scRNA-seq and scATAC-seq data. Furthermore, OTMODE exhibited excellent control of false-positive rates in simulated datasets devoid of differentially expressed features, underscoring its precision. Additionally, OTMODE integrates seamlessly with Scanpy and demonstrates superior performance in computational efficiency and annotation reliability across simulated and real datasets, offering a robust and user-friendly solution for single-cell analysis.

Conclusion: Our study demonstrates that OTMODE effectively addresses these challenges by overcoming data sparsity and cellular heterogeneity while providing a universal framework capable of handling various types of omics data. We believe that the proposed framework has the potential to advance the exploration of complex and nuanced alterations in biological mechanisms across diverse omics contexts.

C.O.I. Disclosure:

H. Su: None. **X. Chen:** None. **W. Yang:** None.

P17.029.B Integrating Multi-Omics and Machine Learning through Polygenic Risk Scores in Middle Eastern Populations for Cardiometabolic Traits

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Background: Cardiometabolic traits like Type 2 Diabetes (T2D) and lipids share complex interrelated pathophysiology. Polygenic risk scores (PRSs) have been developed mainly in European cohorts, often focusing on single traits and lacking multi-omics integration. Here, we evaluated the use of machine learning (ML) to integrate multi-omics data through PRS, and explored the importance of ensemble learning to develop multi-trait PRS in the Middle East.

Material and Methods: Qatar Precision Health Institute dataset was used (13,994 whole genome sequence individuals, 2,906 with metabolomics and 2,845 with proteomics data). We studied 22 cardiometabolic traits (e.g., T2D, Hypertension, LDL-C, etc.). Local PRSs were developed, and 1,072 existing PRSs were evaluated. PRSs were combined linearly (ensemblePRS) or using ML models: support vector machines (SVM), random forests (RF), and xgboost.

Results: 948 of 1,072 PRS were associated with at least one trait ($P < 4.66 \times 10^{-5}$). For example, PGS000020 was the best for T2D (OR = 1.75, CI [1.54–1.99]; AUC = 0.60, CI 95% [0.58–0.63]; $P = 5.14 \times 10^{-18}$) and PGS003871 was the best for LDL-C ($R^2 = 0.12$, CI 95% [0.10–0.14]; $P = 2.91 \times 10^{-137}$). Local PRS slightly improved performance for 9 of 22 traits. EnsemblePRSs outperformed single PRSs (AUC = 0.62 vs. 0.60 for T2D). Xgboost surpassed RF and SVM (AUC = 0.64, 0.60, 0.61 for T2D, respectively). Multi-trait PRSs had an advantage over trait-specific PRSs, especially for lipids. Combining ensemblePRSs and xgboost predictions showed the best performance. Finally, adding omic scores showed marginal improvement for hypertension only.

Conclusion: Public PRSs transfer well to Middle Easterns for many cardiometabolic traits. Multi-trait and trait-specific PRSs combined with ML tools had the best performance.

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A. Shaar: None. **M. Elshrif:** None. **E. Ullah:** None. **G. Nemer:** None. **M. Bashir:** None. **M. Saad:** None.

P17.030.C AI-Driven Automation of Genetic Variant Interpretation: Enhancing Clinical Reporting with Large Language Models

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Background: Interpreting genetic variants and writing structured reports is a time-consuming task that requires synthesizing diverse sources of evidence. Large Language Models (LLMs) are well suited for this challenge, as they can process vast amounts of textual data, extract key insights, and generate structured interpretations efficiently.

Methods: We developed a Python-based computational pipeline integrating Retrieval-Augmented Generation (RAG) and few-shot prompting techniques to enhance the quality of AI-generated interpretations. The system employs multiple specialized AI agents: (1) a literature review agent that retrieves and prioritizes relevant publications via LitVar, with additional fine-tuning based on ClinVar pathogenic variant-associated literature; (2) a clinical feature extraction agent that synthesizes phenotype descriptions from OMIM in regards of patient's HPO terms; and (3) a classification agent that structures the final output based on ACMG/AMP variant interpretation guidelines.

Results: The AI-generated commentaries provide structured and clinically relevant interpretations of genetic variants. Each report consists of three key sections: (1) a description of the variant, including molecular characteristics and inheritance patterns; (2) a synthesis of associated clinical features based on OMIM and related databases; and (3) a proposed classification, integrating genotype-phenotype correlations and literature-based evidence.

Conclusion: By leveraging LLM-powered AI agents and computational algorithms, our approach streamlines the process of variant interpretation and report generation. This enables clinicians to focus on complex cases while benefiting from AI-assisted summaries that ensure consistency and scientific rigor. Moreover, by incorporating curated input data and expert annotations, we enhance the original content generated by human experts, ultimately optimizing time in clinical practice.

C.O.I. Disclosure:

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P17.031.D Transformer-Driven multi-modal integration of phenotypic, facial, and genomic data for optimized rare disease variant prioritization

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Background: Variant prioritisation is a critical step in genomic analysis, crucial for identifying disease-associated genetic variations. Improving the variant prioritization accuracy requires the integration of phenotypic, facial, and genomic data. Existing tools often struggle to effectively combine these diverse data types. This study introduces a transformer-based framework that integrates Human Phenotype Ontology (HPO) terms, facial image embeddings, and genomic variant annotations to enhance variant prioritization.

Material and Methods: The framework processes phenotypic data encoded as HPO terms, facial images converted into embeddings, and genomic variants annotated with scores such as CADD and allele frequencies. These modalities are preprocessed and embedded in a shared latent space. A multi-modal transformer model dynamically weighs their contributions using attention mechanisms, prioritizing variants based on the combined input.

Results: Performance was evaluated using whole-exome sequencing data from 186 patients with rare disorders. The framework achieved improved diagnostic accuracy, outperforming existing tools such as PEDIA and Exomiser. Integration of facial phenotypes improved accuracy in syndromes with facial dysmorphism, while dynamic attention mechanisms enhanced the model's ability to prioritize causative variants in phenotypically diverse cases.

Conclusion: This study demonstrates the potential of transformer architectures to integrate phenotypic, facial, and genomic data for rare disease diagnostics. The framework surpasses current state-of-the-art tools, providing a scalable and accurate method for variant prioritization. Future extensions will explore how the model can adapt to patient-specific features, such as age and ethnicity, to improve prioritization accuracy in diverse populations.

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M. Bhasin: None. **T. Hsieh:** None. **A. Knaus:** None. **P. Krawitz:** None.

P17.032.A Assessing nextflow transcriptomic pipelines for identifying candidate genes associated with neurodevelopmental disorders

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Background: Intellectual disorders (ID) and autism spectrum disorders (ASD) are heterogeneous neurodevelopmental (NDD) conditions with a strong genetic component. RNA-seq analysis is a powerful tool for the identification of aberrant or outlier gene expression and alternative splicing as functional evidence to support rare disease study and diagnosis. Here, we evaluated the effectiveness of Nextflow pipelines in identifying candidate differentially expressed genes in individuals with ID and/or ASD.

Materials and Methods: RNA sequencing (RNA-seq) data from 11 patients with ID and/or ASD and 7 family controls were analyzed using the nf-core *rnaseq* pipeline for quality control and expression quantification. Differential gene expression analysis was performed using the nf-core *differentialabundance* pipeline. Identified DEGs were further investigated through GeneTrek and DisGeNET to assess their relevance to NDD.

Results: The combination of *rnaseq* and *differentialabundance* pipelines allowed the identification of 6 over-expressed and 4 under-expressed protein-coding genes in the patient cohort. According to GeneTrek, among the over-expressed genes, PTK7 was associated with neurodevelopmental disorders with high confidence, while 2 other genes were considered potential candidates for further investigation. For the under-expressed genes, 3 out of 4 are also listed as possible candidates for neurodevelopmental disorders.

Conclusion: Despite the limited sample size, our findings highlight the effectiveness of Nextflow-based pipelines for RNA-seq analysis in identifying candidate genes associated with ID and/or ASD. Notably, PTK7 has been previously linked to neurodevelopmental disorders, while the other differentially expressed genes emerged as possible candidates for further investigation.

List of overexpressed and underexpressed genes

Gene	Adjusted p-value	Log2FC	GeneTrek Lists	Reference
LRRN3	1.58E-04	1.252	Candidate	PMID:2067824
CCR9	2.16E-04	1.195		PMID:17181556
CD248	2.94E-04	1.377		
PTK7	5.33E-03	1.077	HC, SFARI, SysNDD	PMID:28191889
BHLHA15	1.81E-02	1.158	Candidate	
FBLN2	1.81E-02	1.077		
KCP	2.4E-02	−1.049		
RCAN2	2.4E-03	−1.740	Candidate	
NAP1L2	1.9E-03	−1.182	Candidate	
IGFBP3	5.5E-06	−1.643	Candidate	PMID:17547689

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P17.033.B Latent-Variable Models for Integrating Rare and Common Variants in Disease Risk Prediction

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Background: Current genetic models for disease prediction lack a unified framework to integrate both rare and common variants, as these are typically studied independently. This dichotomous approach likely contributes to diagnostic gaps in rare diseases and limits the identification of high-risk individuals for common traits.

Material and Methods: We propose a generative, latent-variable modelling framework to predict the joint impact of common and coding rare variants enabling the discovery of new disease subclasses. Rare variants are collapsed at the gene level using popEVE pathogenicity scores as weights, improving performance while reducing genotype dimensionality. This allows for the inclusion of a large number of rare variants while retaining single-variant information. Phenotype prediction is achieved through a Variational AutoEncoder (VAE) -like architecture that creates a latent representation of the genetic variants. Each individual's genotype is converted to a representation that disentangles common and rare variant contributions and maps their relationship to phenotypic severity.

Results: Using UK Biobank imputed genotypes and exome sequencing data (N ~ 400,000), we applied our framework to highly heterogeneous diseases with unclear diagnostic criteria and frequent comorbidities, including Type 2 Diabetes (T2D), Coronary Artery Disease and Hypertension. Our model significantly improved the identification of high-risk individuals for T2D and related complications compared to existing methods.

Conclusion: We developed a family of models that quantify the contributions of rare and common variants to individual disease risk. Our approach expands the number of patients who can receive a clearer genetic diagnosis, leading to more actionable therapeutic strategies.

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F. Billeci: None. **S. Bonàs Guarch:** None. **J. Frazer:** None. **M. Dias:** None.

P17.034.C Estimating the genetic basis of quantitative traits across populations

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Background: Genome-wide association studies are being disproportionately skewed towards analyses in the European population, which induces health disparities for underrepresented groups and hinders understanding of underlying genetic mechanisms. However, these issues are not resolved by simply increasing the diversity of participants, while applying conventional methodology that does not account for differences in allele frequencies, linkage disequilibrium (LD) patterns, and environmental exposures across populations.

Material and Methods: We propose a multivariate Bayesian approach designed to work on summary statistics and individual-level data, which models effect sizes jointly both across genome and across populations and explicitly utilizes population-specific allele frequencies and LD. We compared the discovery and genomic prediction performance of this approach and other state-of-the-art methods on simulated data and height data from the UK Biobank.

Results: The proposed joint modelling of effect sizes allows for precise estimation of the covariance of effect sizes and testing for shared and population-specific effects. In simulation analyses, we demonstrate how such multivariate modelling leads to improved fine-mapping and cross-group polygenic prediction accuracy. On the real data, the proposed summary statistics model outperforms other existing multi-ancestry methods in prediction accuracy and reaches heritability estimates comparable to the results of the best individual-level models (e.g. 0.67 for height for European ancestry).

Conclusion: We provide empirical evidence that discovery and genomic prediction are greatly enhanced by analyzing effect sizes across genome and populations jointly. Therefore, multivariate analysis incorporating multi-ancestry data should be commonplace, improving our ability to infer shared and population-specific genetic architecture patterns of human complex traits.

C.O.I. Disclosure:

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P17.035.D The shared and differential genetic risk factors of neurodegeneration

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Background: Neurodegenerative diseases comprise many complex pathologies that result in neuronal loss. Heterogeneous in nature, they often exhibit overlapping symptomatologies, protein aggregates, and comorbid psychiatric disorders, resulting in diagnostic difficulties for even experienced neurologists. Recent literature suggests overlapping genetic risk for these diseases, which could be core to the biology of neurodegeneration.

Material and Methods: This research aims to identify the shared genetic risk factors that contribute to nine neurodegenerative traits, namely Alzheimer's Disease, Amyotrophic Lateral Sclerosis, Creutzfeldt-Jakob Disease, Frontotemporal Dementia, Lewy-body Dementia, Multiple Sclerosis, Neuromyelitis Optica, Parkinson's Disease, and Vascular Dementia. We analysed summary statistics from their largest genome-wide association studies (GWAS) using LD-score regression (LDSC) and genomic structural equation modeling (GenomicSEM) to examine genetic correlations between traits and model their complex interrelatedness. To uncover shared SNPs between neurodegenerative disorders, we used multi-trait analysis of GWAS (MTAG) and conjunctional false discovery rate (conjFDR) analysis as complementary methods. Gene ontology analysis was run on positionally mapped genes from significant loci using FUMA.

Results: We identified genetic correlations and a shared latent genetic factor between several neurodegenerative traits. MTAG analyses revealed 129 novel risk loci significantly associated with our nine neurodegenerative traits, 25 of which were reinforced by conjFDR as shared between two or more neurodegenerative traits. These loci were enriched for differential expression in dopaminergic neurons and several gene ontologies relevant to neurodegeneration (e.g. microglial cell activation, neuron death regulation).

Conclusion: Multi-trait analysis of neurodegenerative traits reveals shared genetic risk loci and improves our biological understanding of core neurodegenerative mechanisms.

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P17.036.A Characterising the genetic architecture of thousands of human traits

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Background: Understanding the genetic architecture of health outcomes is important for improving disease risk prediction models and advancing personalised medicine. This includes estimating heritability, polygenicity, importance of selection, as well as evaluating the prediction accuracy of polygenic scores (PGS). In this study we present an efficient and parallelized pipeline that uses LDpred2-auto to efficiently infer the genetic architecture of human traits. We apply this to thousands of traits, creating a comprehensive map of genetic architecture for human diseases and phenotypes.

Material and Methods: GWAS summary statistics were obtained from the GWAS Catalog and filtered based on numerous criteria, including effective sample size (N < 50000) and having at least one genome-wide significant association. After applying LDpred2-auto with the HapMap3+ variants, we further filtered the results based on convergence. The pipeline supports high-performance computing and is publicly available on Github (https://github.com/olex2148/PGS-pipeline).

Results: We downloaded 64592 GWAS summary statistics, after filtering we applied LDpred2-auto to 2030 outcomes. LDpred2-auto converged without issues for 1908 outcomes, where we inferred PGS prediction accuracy R2), polygenicity p, SNP heritability SNP-h2, and selection coefficient α. We grouped these model parameters by trait categories based on ontology terms. When possible we also estimated observed out-of-sample prediction accuracy in the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) cohort.

Conclusion: Our scalable LDpred2-auto pipeline can efficiently infer key parameters for thousands of heritable diseases and phenotypes. Our results provide a comprehensive map of genetic architecture in human health outcomes and provide important insights into disease aetiology and how to develop robust PGSs.

C.O.I. Disclosure:

O. Hansen: None. **J. Grove:** None. **B. Vilhjálmsson:** None.

P17.037.B Next-generation phenotyping facilitates the identification of structural brain malformations in rare disorders through computational brain MRI analysis

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Background: Many rare disorders present with structural brain malformations, posing challenges for neurologists in identifying the "brain gestalt" associated with these conditions. Next-generation phenotyping (NGP), which has successfully recognized facial gestalt of syndromes by training thousands of patient photos, can similarly be applied to brain MRI data. By learning patterns from brain MRIs, NGP can detect structural abnormalities such as lissencephaly. This study introduced a deep learning-based NGP approach to identify brain malformations, providing clinicians with diagnostic support for rare disorders.

Material and Methods: We curated 663 brain MRIs spanning 56 disorders in the GestaltMatcher Database. Using transfer learning with ResNet-50, pre-trained on the fastMRI dataset (6,970 MRIs) for age prediction, we encoded MRIs into feature vectors, creating the "Clinical Brain Phenotype Space (CBPS),"

where proximity reflects phenotypic similarity. Additionally, 1,279 pediatric MRIs from public datasets were encoded as healthy controls. Feature-space distances were used to estimate the probability of associated disorders.

Results: Evaluating Mowat-Wilson syndrome, lissencephaly, tubulinopathy, *RNU4-2*, and Ogden syndrome, CBPS distinguished conditions from controls with an accuracy of 90% via leave-one-out cross-validation. t-SNE visualization showed distinct clusters for these conditions, confirming CBPS's potential for phenotypic clustering and disease prediction.

Conclusion: This study demonstrated the application of NGP to structural brain malformations in rare disorders. While our initial analysis focused on five specific conditions, the results highlight the feasibility of extending this approach to a broader spectrum of genetic disorders.

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P17.039.D A data-driven study to describe the functional landscape of pathogenic genomic variants in a cohort of 11,000 patients with rare diseases

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Background: Rare diseases (RD) are life-limiting conditions with a low prevalence and a large phenotypic and genotypic heterogeneity. Diagnostic rates remain critically low (~50%) and need to be improved. This work employed a data-driven approach to extract global and specific genotype-phenotype associations in RDs.

Material and Methods: Genomic variants from a cohort of >11,000 patients with >125 RDs were studied using a common bioinformatics framework. Using case-control comparisons, we ranked the most frequent pathogenic variants (MFPVs) across ~30 groups of related diseases (GRDs). For each GRD, Gene Set Enrichment Analysis (GSEA) was performed on 1) the ranked MFPVs and on 2) the ranked genes based on their MFPVs' burden to extract associated functions (Interpro, Gene Ontology, Reactome, KEGG) and phenotypes (HPO). The GRDs were then analyzed collectively within a similarity network.

Results: We present ranked predicted pathogenic variants associated with each GRD, identifying 980 protein domains enriched in MFPVs across 37 GRDs. For each GRD we ranked genes based on their MFPVs' burden and provide a comprehensive overview of the biological processes, potentially affected pathways, and the resulting phenotypes. Additionally, we conducted a global analysis of shared and GRD-specific functions and phenotypes and we discussed a network of GRDs linked by their functional and phenotypic similarities. Finally, we wrapped these results into a prioritization strategy applied in the reanalysis of non-solved cases.

Conclusion: We report a global survey of variants, genes, functions and phenotypes associated to >125 RDs using a hypothesis-free strategy that feeds guided reanalysis of RD non-solved cases.

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P17.040.A Generative Machine Learning approach to generate genotypes and phenotypes for privacy-preserving sharing of polygenic risk prediction data

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Background/Objectives: Polygenic Risk Scores (PRSs) rely on GWAS summary statistics, which exclude non-linear SNP-SNP interactions. Machine Learning (ML) can model these effects and improve PRS performance, but requires large raw datasets, often inaccessible and difficult to combine due to privacy concerns. We propose a generativeAI method to create synthetic data, enabling privacy-preserving sharing and integration of raw data.

Methods: We trained a Latent Diffusion Model conditioned on a binary outcome in a simulated scenario: a disease with 20% prevalence determined by 2048 real SNPs. Generated data quality was assessed by comparing SNP correlation structure and concordance of GWAS betas between synthetic and real data, while verifying that no synthetic samples replicated real genotypes to assess privacy. Finally, predictive performance was tested by training ML models and canonical PRSs on generated or real datasets using Cross-Validation. We conducted the same experiments on real phenotypes, including Coronary Artery Disease (CAD) and Breast Cancer (BC) cases and controls from UK biobank.

Results: In simulated and real case scenarios, betas computed from synthetic and real data showed strong correlation (>0.75) while preserving SNP correlation structure and privacy. Cross-Validation showed that ML models trained on synthetic data performed comparably to canonical PRSs on real data.

Conclusions: We have shown that it is possible to generate realistic synthetic genotypic and phenotypic data that retain the information content of the training set while preserving participants' privacy. This enables the integration of restricted-access data for training powerful ML risk models, overcoming the limitations of current sumstats based PRSs.

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P17.041.B Deep Learning Based Integration of MRI Radiomics and Multi-Omics for Medulloblastoma Subtyping and Prognosis prediction

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Abstract

Background: Radiogenomics, which integrates imaging features with multi-omics data, provides a novel approach for understanding tumor heterogeneity and improving clinical decision-making. In medulloblastoma, a highly heterogeneous brain tumor, the correlation between MRI-based radiomic features and molecular characteristics remains underexplored. This study aims to develop imaging-driven biomarkers and predictive models for tumor subtype classification, prognosis assessment, and metastasis prediction using deep-learning approaches.

Materials and Methods: Multi-omics data, including methylation profiling, whole-genome sequencing (WGS), RNA sequencing (RNA-seq), single-cell ATAC-seq (snATAC), and spatial transcriptomics, were collected from multiple cohorts (Tiantan Cohort, EGA Cohort, ShijiTan Cohort). A structured workflow was designed, incorporating MRI-based methylation prediction, radiomic feature extraction from high-interest regions, deep-learning-based image-molecular association analysis, and validation of imaging biomarkers in patient stratification and prognostic modeling.

Results: MRI-based radiogenomic biomarkers successfully distinguished medulloblastoma subtypes, demonstrating strong correlations with molecular classifications. The integration of imaging and methylation data improved prognosis prediction, providing a non-invasive method for outcome assessment. Additionally, MRI features were significantly associated with metastasis-related molecular changes. Deep-learning models outperformed traditional approaches in imaging-molecular correlation analysis, enhancing the accuracy and reliability of tumor classification and risk prediction.

Conclusion: This study establishes a deep-learning-driven radiogenomic framework for medulloblastoma, offering a non-invasive strategy for tumor subtype classification, prognosis evaluation, and metastasis prediction. The findings highlight the potential of integrating MRI imaging with multi-omics data to advance precision oncology and facilitate personalized treatment strategies.

C.O.I. Disclosure:

Z. Wang: None. **X. Nie:** None.

P17.042.C A Bayesian FPCA framework for modelling high-dimensional biomarker trajectories

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Background: The increasing availability of longitudinal measurements on large panels of gene products is set to improve our understanding of the molecular processes underlying disease risk and progression. While functional data analysis is an active area of research, methods for modelling complex multivariate functional dependencies remain limited.

Material and Methods: Motivated by a COVID-19 study conducted in Cambridge, we propose a Bayesian approach for representing high-dimensional biomarker curves, combining latent factor modelling and functional principal component analysis (FPCA). This approach captures correlations across biomarkers and time by positing that subsets of biomarkers contribute to a small number of FPCA expansions (e.g., representing latent disease processes) through biomarker-specific loadings. Subject variability is modelled using a small number of functional principal components, each characterised by a smoothly varying temporal function. We develop a variational inference algorithm, with analytical updates, that couples efficiency and principled parameter uncertainty quantification, and we propose strategies to learn the number of factors and FPCA components involved in each factor's expansion.

Results: Extensive numerical experiments illustrate the ability of the approach to (i) accurately estimate biomarker-specific loadings, FPCA latent functions and subject-specific component scores, and (ii) scale to high-dimensional datasets, e.g., with panels of 20,000 genes measured longitudinally for a few hundred subjects.

Conclusion: In the COVID-19 study, our framework helped disentangle disease heterogeneity and clarified which biomarkers coordinate over time, highlighting key biological pathways. More broadly, our approach also enables prediction of biomarker trajectories at the subject level, towards personalised treatments in a variety of disease contexts.

C.O.I. Disclosure:

S. Jaoua: None. **D. Temko:** None. **H. Ruffieux:** None.

P17.043.D Analysis strategy for whole genome data in a national genome project in korea

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Background: Efficient analysis of large-scale whole genome sequencing (WGS) data is crucial for advancing population genetics and identifying disease associations. Comprehensive strategies for WGS data can uncover environmental factors contributing to disease susceptibility, enabling tailored preventive measures and therapeutic interventions. Accumulating genomic data drives precision medicine, medical technology progress, and improved public health outcomes.

Material and Methods: We developed efficient strategies for germline and somatic variant calls, joint genotype analyses, and WGS-based gender and familial relationship analyses, tailored to user environments. Optimal CPU and memory configurations for GATK-based pipelines were explored, and a structured data storage system was established to ensure efficient data access and management.

Results: The strategies provided consistent analysis capacity regardless of scale, optimizing resource allocation. Enhanced CPU and memory configurations improved pipeline efficiency, while structured storage simplified data management. These results strengthened the scalability and reliability of WGS analyses under resource constraints.

Conclusion: This study optimizes the WGS analysis framework by significantly reducing processing times for germline, somatic, and joint genotyping. Furthermore, detailed specifications of required computational resources were provided, enabling users to easily implement and adapt the framework. These advancements prioritize user accessibility and efficiency, ensuring a streamlined approach to large-scale WGS data analysis.

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P17.045.B Developing machine learning models for predicting cytochrome P450 ligand potency

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Background: Cytochrome P450 (CYP P450) enzymes are involved in over 90% of known metabolic reactions. To understand the

interaction between CYP P450 enzymes and their ligands, a variety of experiments have been performed including in silico methods such as machine learning (ML) predictions. ML models can predict the potential interactions of enzymes with ligands of interest.

Material and Methods: In this study, we developed ML models based on several algorithms including the Support Vector Machine, Extreme Gradient Boosting (XGBoost), Random Forest, Neural-network and K-Nearest Neighbor. The models serve to predict the potency of ligands with respect to CYP P450 enzyme sequences. To develop the models, over 30 000 enzyme assay data was acquired from BindingDB. From the data, CYP P450 enzyme sequences were encoded using AAindex indices and the ligands were encoded using molecular descriptors, which were generated using RDkit and Mordred. In addition, the IC50 values of the enzyme assays were also encoded.

Results: Dimensionality reduction was performed on the enzyme and ligand features prior to training the models. The performance of the models was assessed using accuracy scores and the Area Under the Curve (AUC) of the Receiver of Operating Characteristic curves. The best performing algorithm was XGBoost with an accuracy of 0.8 and an AUC score of 0.9.

Conclusion: This indicates that machine learning can be used to predict the potency of ligands with respect to enzymes of interest. Such models can be potentially employed for drug discovery, protein engineering and pharmacogenetics.

C.O.I. Disclosure:

B.B. Sitabule: None. **S.S. Hazelhurst:** None. **H.H. Othman:** None.

P17.046.C Mapping the mitochondrial RNA modification landscape using Nanopore sequencing

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Background: N1-methyladenosine (m1A) modification in mitochondrial RNA (mtRNA) has emerged as a potential regulatory factor in neurodegenerative diseases, particularly Alzheimer's disease. Despite its potential significance, the distribution and dynamics of m1A modifications in disease states remain poorly understood, hindering accurate diagnostics and understanding of the disease. Thus, there is a strong need for novel analyses using Nanopore sequencing.

Material and Methods: We employed Nanopore sequencing to analyze mtRNA from Alzheimer's disease patients and age-matched controls. This technology enables direct detection of RNA modifications through characteristic current signal perturbations as native RNA molecules traverse the nanopore. A novel neural network-based detection tool was developed and trained on synthetic oligonucleotides containing known m1A sites. The analysis pipeline integrates multiple bioinformatic tools and custom visualization methods for comprehensive modification mapping.

Results: We profiled the RNA modification landscape of mtRNA from healthy human blood samples, establishing baseline modification patterns. Initial analyses demonstrate successful detection and mapping of m1A modifications across mitochondrial transcripts with high confidence. Systematic benchmarking of our neural network approach against existing tools shows improved detection accuracy. Comparative analysis revealed distinct m1A distribution patterns between patient and control groups.

Conclusion: While multiple RNA modifications exist in mtRNA, current detection tools show considerable variability in their results. Despite these challenges, Nanopore sequencing proves to be a valuable tool for direct RNA modification detection. We establish a robust framework for analyzing mitochondrial RNA modifications in neurodegenerative diseases, aiming to identify novel biomarkers for early disease detection and monitoring.

C.O.I. Disclosure:

L. Lehmann: None. **L. Walz:** None. **K. Friedland:** None. **S. Gerber:** None.

P17.047.D Resources for structural variant interpretation: Human Genome Structural Variation Consortium data and the Ensembl Variant Effect Predictor

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Background: The accurate identification and annotation of structural variants (SVs) is essential for genomic research and clinical variant interpretation. The Human Genome Structural Variation Consortium (HGSVC) exists to create accurate catalogues of precisely defined SVs and reusable methods for SV characterisation, interpretation and visualisation.

Material and Methods: To facilitate discovery, we have integrated HGSVC data into the International Genome Sample Resource (IGSR), a FAIR resource for genomic data sharing. To accelerate SV interpretation, we have extended the Ensembl Variant Effect Predictor (Ensembl VEP) functionalities.

Results: The IGSR data portal enables the discovery and download of HGSVC datasets, filtering by data type, sample or technology. The latest release includes a catalogue of precisely defined SVs from the haplotype-resolved assemblies of 65 individuals of diverse ancestry. These near-complete assemblies provide a more detailed insight into human genome variation. Ensembl VEP annotates SVs with the genes they overlap and predicts likely impact. Further information on gene function, reported phenotype association and measures of tolerance to loss of function variation, hyposensitivity and triplosensitivity are reported to aid interpretation.

Conclusion: These resources demonstrate advances in SV resolution and annotation which can help advance understanding of disease association and mechanism which could accelerate disease diagnosis.

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P17.048.A Validation of identity-by-descent tools applied to whole-exome sequencing data

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Background: In many studies, it is important to know the degree of relatedness between participants. Most current tools for relatedness detection are primarily designed for SNP array or whole-genome sequencing data. This poses a challenge for labs relying on whole-exome sequencing (WES), as it remains unclear how these tools perform with WES data. We investigated the use

of WES data to uncover close and distant relationships by validating four identity-by-descent (IBD) tools: TRIBES, GRAPE, XIBD and DRUID.

Material and Methods: We included 30 individuals from a Norwegian family with a known tenth-generation pedigree and 30 presumed unrelated individuals from a Norwegian ALS cohort. All participants had provided a written informed consent. All samples underwent WES and were processed with an inhouse nextflow pipeline to produce a quality-filtered jointvcf. Using this vcf as input, we applied each IBD tool to estimate all 1770 pairwise relationships between the 60 individuals.

Results: Up to 5th degree, two of the tools exhibited strong precision and recall: TRIBES and GRAPE both matched exactly for degree 1 and 2, a majority of exact matches for degree 3 and within one degree for 4th and 5th degree. DRUID differed in that it produced very few exact matches, but matched within one to two degrees. For 6th degree relationships, TRIBES was the only tool exhibiting a majority of exact matches. Updated results and XIBD results will be presented at the meeting.

Conclusion: Whole-exome sequencing data for detection of relatedness up to 6th degree is feasible utilizing IBD tools.

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P17.049.B KRAS-Driven Immune System Modulation in Pancreatic Cancer: Insights from Exosome-Mediated KRAS Inhibition

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Background: *KRAS* mutations drive pancreatic ductal adenocarcinoma(PDAC) progression, yet their downstream effectors in immune evasion remain poorly characterized. While *KRAS*-driven immunomodulation has been studied, involvement of *TRIB1* and *RCAN1* in this process has not been reported. We investigated these genes as potential mediators of *KRAS*-dependent immunosuppression.

Material and Methods: We analyzed the GSE97594 dataset, where PANC-1 cells(*KRAS*G12D mutant) were treated with *KRAS*-targeting siRNA-loaded exosomes(iExosomes). Additionally, expression levels of *TRIB1* and *RCAN1* were compared between *KRAS*-mutant PANC-1 and *KRAS*-wildtype BxPC3. Differentially expressed genes were validated using Enrichr, TCGA-PAAD for clinical correlation, and Kaplan-Meier survival analysis. Immune infiltration analysis performed using TIMER and MCP-COUNTER.

Results: TRIB1 and RCAN1 were overexpressed in KRAS-mutant PANC-1 and upregulated in TCGA-PAAD tumor samples compared to *KRAS*-wildtype BxPC3. iExosomes-mediated *KRAS* inhibition significantly downregulated *TRIB1* and *RCAN1*. Pathway enrichment linked *TRIB1* to immune system-related signaling(NF-κB,IL-2/STAT5,TGF-β pathways) and *KRAS* signaling.*TRIB1* strongly correlated with *KRAS* expression, while *RCAN1* showed a weak association. High *TRIB1* and *RCAN1* expression predicted worse overall survival.*TRIB1* negatively correlated with CD8+ T cells and CD8+ effector memory cells. *RCAN1* positively correlated with CD8+ T cell infiltration.

Conclusion: Our findings for the first time reveals *TRIB1* and *RCAN1* as *KRAS*-driven immunomodulators in PDAC. Exosome-mediated *KRAS* inhibition downregulates *TRIB1*, suggesting its potential as a therapeutic target. *TRIB1's* strong prognostic value supports its role in clinical risk stratification and immunotherapy response prediction. Immune infiltration analysis suggests that *RCAN1* is associated with CD8+ T cell infiltration, while *TRIB1* may contribute to T cell dysfunction, reinforcing their roles in *KRAS*-mediated immunomodulation.

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P17.050.C From sequencer to analysis: Establishment of an open-source, fully automated, scalable, database-integrated next-generation sequencing pre-processing framework

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Background: From novel short-read sequencing technologies to the rapidly evolving field of long-read sequencing, the bioinformatic processing of genetic data has never been more diverse and demanding. Solutions that cater to the individual sequencers often lead to a series of independent scripts and pipelines, over time resulting in an IT infrastructure that is error-prone and associated with high maintenance costs. To conquer this challenge, there is a need for an automated, scalable, and adaptive bioinformatics framework, enabling a reliable and quick execution of routine operations across different sequencers.

Material and Methods: We developed a Python framework that currently encompasses ten intertwined but independently usable main modules. One of which continuously monitors the user-configured sequencers to identify new sequencing runs and to automatically start their processing. Furthermore, the framework closely interacts with the LIMS (GEPADO XPro) via API to automatically extract the metadata from samples and return processed sample information such as quality control parameters on a run and sample level.

Results: Our bioinformatics pre-processing pipeline unites automated sample sheet creation, data management, demultiplexing, and quality control of raw and processed data. The communication with the LIMS database enables the real-time monitoring of the sequencing run during processing and the modularized composition of the framework allows for the future implementation of new features, such as individualized downstream sample processing.

Conclusion: We developed an open-source, fully automated, scalable, database-integrated next-generation sequencing pre-processing pipeline, which automatically identifies completed sequencing runs on various sequencing devices and prepares them for further downstream processing.

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P17.051.D Prediction of genes associated with Autism Spectrum Disorder using sequence and graph embedding

methods

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Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder, characterized by a strong genetic component, heterogeneous phenotypic presentation and complex genetic architecture. Identification of ASD risk genes and biological pathways is key to understanding this disorder. Despite the large number of genes associated with ASD, its genetic etiology is still not fully understood. To address this challenge, we present a machine-learning approach for ASD risk gene prediction.

Material and Methods: We used gene representations based on graph and sequence embeddings, from protein-protein interaction (PPI) networks and from gene DNA and protein sequences, respectively, with supervised Machine Learning (ML) classifiers. Graph embeddings were produced with the Grape Python library and the STRING PPI database, protein embeddings were obtained with the ProtT5 model and gene embeddings with DNABERT-2. The list of ASD associated genes used to train the classification models was derived from the SFARI database

Results: We developed a Machine Learning-based approach to identify and rank ASD associated genes. Using a 5-fold nested stratified cross-validation, our method achieved an AUC of 0.90, F1 of 0.82, and MCC of 0.77. Our approach compares favorably with state-of-the-art approaches in identifying genes targeted by loss-of-function (LOF) mutations in the MSSNG and Simons Simplex Collection cohorts. Analysis of the network spanned by the top decile genes identified four communities, significantly enriched for biological pathways associated with ASD, including Chromatin Organization and Synaptic Signaling.

Conclusion: This study improves existing ASD risk gene predictions and brings us a step closer to better understanding this complex genetic disorder.

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P17.052.A Structure, sequence and evolutionary relationships in the phenotypic effects of ABAT mutations

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Background: The ABAT gene encodes aminotransferases involved in reversible transamination reactions, including class III aminotransferases such as GABA transaminase (GABA-T). Mutations in ABAT (16p13.2), though rare, are linked to neonatal encephalopathy and psychomotor delay due to GABA-T deficiency. This study investigates structural correlations between ABAT mutations and clinical manifestations.

Material and Methods: Public databases like UniProt were used to obtain ABAT sequences classified as benign or pathogenic. PFstats identified conserved or co-evolving positions, and structural analyses were performed using PyMOL and Modeller to assess mutation effects on protein interactions, physicochemical properties, and structural location.

Results: Eighteen variants were selected (5 benign, 12 pathogenic, 1 VUS). The R19C variant, located in the signal peptide region, was excluded, resulting in 17 models based on the wild-type 1OHV structure. Six variants showed hydrophobic exposure discrepancies: Q56L (benign) had an unfavorable substitution, while R92Q, L487P, L211F, R220K, and L478P exhibited conservative substitutions. The V479F (VUS) mutation also displayed a conservative surface substitution. Interaction analysis revealed that G465D and G465S had increased interactions with R435, whereas six variants (R92Q, Y426, G478S, H484Q, L487P, and L487Q*) showed reduced molecular interactions, affecting different residues.

Conclusion: These findings suggest that specific ABAT mutations impact protein stability and interactions, potentially influencing their pathogenicity. Variants with altered hydrophobic exposure or molecular interactions may disrupt structural integrity. Further studies are needed to correlate these structural changes with clinical phenotypes.

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L. Melo de Jesus: None. **L. Bleicher:** None. **M. Freire dos Reis:** None.

P17.053.B Integration of common and ultra-rare variant burden to identify molecular subnetworks linked to membrane electrophysiology in genetic generalized epilepsy

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Background: Common and ultra-rare variants are contributing risk factors for different epilepsy subtypes, particularly genetic generalized epilepsy (GGE). However, the extent to which these variants underlie the molecular mechanisms related to GGE pathogenesis has not been well understood. This study aims to identify gene networks and biological processes with potential association with GGE, along with candidate driver genes.

Material and Methods: We have integrated GGE-associated common variants from the ILAE Consortium's latest genome-wide association study with ultra-rare variants from whole-exome sequencing of 5,499 individuals by Epi25 Collaborative. The Burden of common and ultra-rare variants was integrated within a molecular interaction network using the NetColoc framework, which relies on a dual network propagation approach.

Results: Our work confirmed that common and ultra-rare variant burden associated with GGE was enriched in gene subnetworks representing epilepsy-related biological processes, including membrane electrophysiology, cell trafficking, and chromatin organization. Additionally, The GGE-associated variants, both common and ultra-rare, were enriched in subnetworks of genes with higher expression in inhibitory neurons compared to excitatory cells. Ultimately, we highlighted putative GGE-related genes, such as *USP8* and *SULT1A2*. These genes have not reached a genome-wide significance level for association with GGE, nor have they been reported as monogenic epilepsy-related genes. However, they show strong colocalization in subnetworks built under the influence of both common and ultra-rare variants.

Conclusion: This work has identified potential candidate genes and underscored the importance of biological processes, notably membrane electrophysiology, in GGE pathogenesis. Our findings can pinpoint new targets for therapeutic intervention and mechanistic studies.

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P17.054.C Alignment to T2T-CHM13 reference genome solves reference mapping bias and improves read mapping at clinically relevant variants

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Background: The Telomere-to-Telomere (T2T) CHM13 reference genome demonstrates large-scale differences with previous human genome assemblies. We investigated whether the T2T assembly improves allelic variation analysis and signal detection from regulatory regions with respect to hg38.

Material and Methods: DNA sequencing human and cell line data from multiple designs (whole-genome, whole-exome, and targeted sequencing) at varying coverages (30x-1000x) were aligned to T2T-CHM13v2.0 and hg38 assemblies. Pileups at high minor allele frequency dbSNP sites were used to call heterozygous genotypes (variant allele fraction (VAF) between 0.2 and 0.8) on a sample basis to quantify the inflation of reference allele counts, the so-called reference mapping bias (RMB). UCSC repository ClinVar, CpG islands, and ENCODE TFBS tracks for hg38 and T2T reference genomes were analyzed through pairwise sequence alignment.

Results: Heterozygous SNP VAF distribution from T2T aligned data showed a significantly reduced RMB compared to hg38 data. We detected mismatches in the genomic sequence in the proximity (±150 bp) of SNPs with discordant VAFs across the two assemblies, suggesting that T2T resolves genomic inaccuracies in bases proximal to SNPs. Genomic differences within ChIP-seq peaks and CpG islands showed negligible impact on TFBS enrichment analyses and methylation calling. Focusing on ClinVar annotated sites, T2T improved read coverage and mapping quality metrics at clinically relevant variants compared to hg38, including pathogenic variants in *BRCA1* exon 17.

Conclusion: The alignment to T2T assembly solves RMB and improves read mapping at disease-associated genomic sites, possibly facilitating the detection of variants for precision medicine testing.

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P17.055.D Interpretable neural network polygenic risk scores for common diseases

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Background: Genetic contributions to complex human phenotypes like risks for common diseases are typically modelled by polygenic risk scores. While they perform well within cohorts, they assume independent variant effects and are thus not suited to model non-linear phenomena like epistasis or group-specific effects that depend on integration of covariates like sex or genetic principal components.

Materials and Methods: We present sparse neural networks utilizing prior knowledge from the Gene Ontology that integrate genotypes and covariates to predict disease risks. Our models are trained on UK-Biobank data and evaluated on the All-of-Us Cohort, together with polygenic risk score baselines that we derived based on UK-Biobank summary statistics and published scores from the PGS Catalog, using the same covariates as in our models.

Results: Our models match or outperform baselines for most of the phenotypes investigated, despite being trained on only a single cohort, the UK-Biobank. For diabetes type 2 specifically, we observe a ROC AUC of 0.605 of our model versus 0.581 for PGS000014 from the PGS Catalog. Furthermore, the proposed models are inherently interpretable since each neuron is mapped to a specific Gene Ontology term, and we observe that terms more important for the models also have higher relevance for the predicted phenotypes.

Conclusion: Using neural networks, we do not assume independence of variant effects, and can better integrate covariates, which leads to improved polygenic risk prediction across cohorts. While our approach introduces challenges like the requirement for access to individual-level data it enables modelling nonlinear effects at scale.

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P17.056.A Seasonal human proteome: a population-based cohort study from South Asia

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Background: Chronic diseases account for 55–82% of deaths in South Asia, increasingly exacerbated by climate change. Proteins provide a dynamic and objective measure of how individuals' health responds to environmental changes. This study aims to identify seasonal plasma protein patterns and underlying environmental impacts on chronic disease.

Material and Methods: We analysed cross-sectional data from a South Asian cohort (N = 10,000), including plasma proteomic 7kSomaScanAssay. Individuals were grouped by blood-sampling date (2016–2019) to generate a seasonal profile dataset of 52 weekly values, balanced for sex, age, BMI and smoking. Protein weekly means and confidence intervals were estimated through bootstrap resampling (N = 1,000), and seasonality modelled by functional data analysis. We probed the role of seasonal proteins on disease through genetically-informed causal inference methods such as colocalization and Mendelian Randomization.

Results: We identified 24% (1535/6368) of the proteome as seasonal using the autocorrelation function. The seasonal proteome grouped into three data-driven clusters peaking before (N = 519), during (N = 557), and after (N = 459) the monsoon season. Seasonal proteins were more likely involved in active secretion and tissue damage, and less in cell leakage compared to non-seasonal proteins, suggesting a broad impact on biological processes. Preliminary results suggest some seasonal proteins causally contribute to cardiovascular and respiratory diseases, while others reflect disease consequences.

Conclusion: This study identified environmental signals in plasma proteins linked to chronic disease, marking the first step

toward targeting modifiable factors for public health interventions in a climate-affected country.

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P17.057.B Multiple Instance Learning applied to population scale single-cell transcriptomics

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The extension of the human lifespan increases the burden of chronic and inflammatory diseases, but the molecular processes behind their heterogeneity are often unknown. Leveraging Electronic Health Records, genotype data, and single-cell RNA-seq, we aim to identify disease-associated cell states, uncover disease heterogeneity, and highlight shared cell states or gene programs across distinct diseases. This approach may guide target prioritization and drug repositioning.

We employ an attention-based multiple instance learning (MIL) framework, MixMIL, on single-cell transcriptomics to identify predictive cell states involved in diseases. First, we benchmark MixMIL using various dimensions of scVI space on a simulated dataset to determine optimal input configurations. The best-performing setup is then applied to the UK-Biobank Cardinal cohort (N = 5000 single-cell RNA-seq PBMC donors).

MixMIL achieves a good performance with 30 scVI latent factors as input, yielding an accuracy of 75%, area under the precision-recall curve of 0.91, and f1-score of 0.73 on the synthetic dataset for the prediction of the simulated class. In the Cardinal cohort, we will apply the same framework to predict 90 disease outcomes, then select the top attended cells and cluster individuals based on cell type proportions among these important cells. Individuals with varying proportions of key cells could exhibit distinct comorbidities, genetic risks (e.g., polygenic risk scores), or environmental exposures.

Our findings underscore MixMIL's utility in pinpointing disease-relevant cell states. In the future, we will benchmark other MIL methods, e.g. MultiMIL. Further analyses are essential to advance our understanding of disease mechanisms and therapeutic opportunities.

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P17.059.D Sawfish: Integrating copy number segmentation with structural variant haplotype modeling to improve large-variant calling accuracy

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Background: Sawfish is a structural variant (SV) caller for mapped high-quality long reads. It leverages SV haplotype assembly and haplotype representation in all downstream sample merging and genotyping steps to improve accuracy. Further improvements to sawfish are attainable by fully integrating depth-based segmentation with the existing SV haplotype model to enrich modeling of large and complex events.

Results: Sawfish has the highest accuracy on the GIAB draft T2T-HG002-Q100 SV benchmark[1] compared to pbsv[2] and Sniffles2[3]. For HiFi WGS 10x depth input, sawfish's F1-score is 0.940 compared to 0.871 and 0.920 for pbsv and Sniffles2, respectively. This accuracy rank persists at all tested depths up to 32x, across all SV size categories, and for SVs comprising the GIAB CMRG benchmark[4].

Sawfish also substantially improves on joint-genotyping accuracy. Genotype concordance was assessed against the known inheritance pattern for seven HiFi WGS samples comprising the 2nd and 3rd generations of CEPH-1463[5]. Here, sawfish calls 28,627 concordant and 3,987 discordant SV alleles (87.8% concordance), greatly improving on both concordant allele count and concordance rate of the next most concordant method, pbsv, with 23,629 concordant and 6,770 discordant alleles (77.7% concordance).

Fully integrating depth-based segmentation within sawfish shows that accuracy is improved by: 1) recharacterizing large breakend-based deletions and duplications which lack corresponding depth signatures, 2) filtering small depth-based calls lacking corresponding breakend signatures, and 3) allowing breakends to guide depth segmentation. This integration additionally allows all depth and breakend-based evidence to be harmonized into a consistent view of all large variants in the sample.

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P17.060.A Inverse probability weighting reduces selection bias in a large-scale genomic cohort of Danish blood donors

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Background: Genomic cohort studies are key for novel discoveries, yet often biased by selection, as exemplified by the Danish Blood Donor Study (DBDS)—a nationwide genomic cohort comprising 140,000 individuals who are disproportionately younger, more likely to be female, more socioeconomically advantaged, and healthier than the general Danish population. Addressing selection bias to account for these systematic differences in genetic research therefore emerges as a pivotal challenge.

Material and Methods: Utilizing comprehensive national registry data, we employed a logistic lasso regression model to estimate inverse probability (IP) weights for all DBDS participants, thereby adjusting for selection bias. This allowed us to conduct IP-weighted GWA studies to evaluate how weighting affects SNP-level effects, genetic correlations, heritability estimates, and Mendelian randomization (MR) estimates.

Results: Selection bias in the DBDS cohort was substantially reduced through IP weighting, creating a pseudo-population closely resembling the general Danish population and reducing bias by nearly 90%. Weighted GWA studies revealed shifts in SNP-level effects, with some associations attenuated or amplified compared to unweighted analyses, while genetic correlation and heritability estimates shifted across multiple traits. Additionally, weighted analyses led to differences in MR-derived causal effect estimates, further underscoring the impact of selection bias on genetic evidence.

Conclusion: Applying IP weighting to the DBDS cohort effectively reduces selection bias, making it more representative of the general Danish population. Our findings highlight the necessity of addressing selection bias in genetic research to improve the accuracy and validity of genetic analyses.

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P17.062.C Unveiling the Contribution of Rare Variants to Breast Cancer Risk using A Bayesian Hierarchical Model

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Background: Breast cancer (BC) is a complex genetic disease. The association at the variant-level of rare variants (RVs, MAF < 0.01) with BC risk in population studies poses challenges due to low statistical power and multiple testing burdens. Thus, current approaches focus on aggregated RVs in high-penetrance genes and known pathways. To investigate the single variant association to BC risk, and improve the interpretability and clinical utility, we propose an alternative approach: the Bayesian Hierarchical Generalized Linear Model (BhGLM).

Material and Methods: We trained and tested the BhGLM using the UK Biobank (UKBB) cohort (15868 BC cases, 165067 controls). We evaluated the identified RVs quality using ACMG and ClinVar annotations, and we built a RVscore embedding the cumulative impact of RVs in BC risk stratification.

Results: The BhGLM approach demonstrated high specificity levels (average FPR ≤ 0.001) in simulation settings. When evaluated on a short list of genes, BhGLM mostly selected pathogenic RVs with higher OR. When extended to the ClinicalExome (5369 genes), we identified a total of 550 LoF RVs in 494 genes. Notably, high levels of the RVScore were associated with an incremented risk of BC compared to PRS alone.

Conclusion: The Bayesan-based approach, not only introduces a novel methodological framework in the context of BC studies, but also enables the quantification of the collective impact of RVs on BC risk while preserving the capacity to interpret the contribution of individual variants.

Table 1. OR for RVScore across PRS classes

PRS class	Covariates	OR (95%CI)	P-Value	N Samples
Low PRS <33%	No Mut	0.54 (0.52–0.56)	< 0.001	11.429
	High RVScore	0.63 (0.58–0.68)	< 0.001	947
Medium PRS 33–66%	No Mut	1 (0.97–1.03)	1	11.422
	High RVScore	1.17 (1.08–1.26)	< 0.001	977
High PRS >66%	No Mut	2.06 (2–2.12)	< 0.001	11.435
	High RVScore	2.4 (2.22–2.59)	< 0.001	966

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P17.063.D Large-scale case-control likelihood ratios to refine variant classification in breast cancer risk genes

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Background: While some variants in susceptibility genes are associated with a substantially increased risk of breast cancer, the impact of a large proportion of variants on risk is still unclear. Such variants, known as variants of uncertain significance, pose

challenges in clinical decision-making, and novel methodologies and data are needed to enhance classification rates.

Material and Methods: A case-control likelihood ratio approach was applied to obtain evidence for classification of rare variants in *CHEK2*, *ATM*, *TP53* and *PALB2*. Sequencing data from over 60K breast cancer cases and over 250K controls from the BRIDGES study of the Breast Cancer Association Consortium and the UK Biobank were used. Likelihood ratios for over 3K variants were computed and results were aligned following ACMG/AMP evidence thresholds. Sensitivity analyses using series of different age-specific penetrance estimates and different number of minimum carriers for each variant were carried out.

Results: Case-control likelihood ratio results showed consistency for variants classified in ClinVar. Using the criterion of variants reaching at least moderate evidence towards or against pathogenicity, the sensitivities and specificities were 66.7% and 100% (*CHEK2*), 88.9% and 81.4% (*ATM*), 79.2% and 94.6% (*PALB2*), and 83.3% and 97.2% (*TP53*). Across the four genes, likelihood ratio evidence in favour, or against, pathogenicity was obtained for an additional 334 rare variants currently annotated as variants of uncertain significance in ClinVar.

Conclusions: The case-control likelihood ratio approach applied to a large-scale sequencing dataset can improve variant classification in breast cancer risk genes.

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P17.064.A A Hitchhiker guide to Structural Variant (SVs) Calling: a comprehensive benchmark through short-reads (srWGS) and long-reads (lrWGS) sequencing technologies

Giuseppe Giovanni Nardone[1], *Valentina Andrioletti*[2], *Aurora Santin*[3,1], *Anna Morgan*[3], *Beatrice Spedicati*[1,3], *Maria Pina Concas*[3], *Ivan Limongelli*[2], *Giorgia Girotto*[1]

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Background: With the advent of new sequencing technologies, SVs identification from Whole Genome Sequencing has become a central topic. However, variability in SVs calling performances persists due to the diversity of available tools and lack of standardized practices. Considering that, we performed a comprehensive benchmark to establish the best performing methodologies for different sequencing technologies.

Material and Methods: Deletions of the HG002 reference sample were evaluated. Benchmark was performed also on low complexity regions (LCR), comparing hg38 to a multi-genome. Several SVs calling tools in combination with different alignment software were evaluated for different sequencing technologies:

Software	Software type	Sequencing technology	Reference
DRAGENv4.2	SVs calling	srWGS	PMID: 39455800
DRAGENv4.0	SVs calling	srWGS	PMID: 39455800
Manta	SVs calling	srWGS	PMID: 26647377
MATCHCLIP	SVs calling	srWGS	PMID: 23967014
DELLY	SVs calling	srWGS	PMID: 22962449
Lumpy	SVs calling	srWGS	PMID: 24970577
SoftSV	SVs calling	srWGS	PMID: 25998133
inGAP	SVs calling	srWGS	PMID: 21715388
Wham	SVs calling	srWGS	PMID: 26625158
CNVnator	SVs calling	srWGS	PMID: 21324876
Cutesv	SVs calling	lrWGS	PMID: 35751813
Nanovar	SVs calling	lrWGS	PMID: 32127024
dysgu	SVs calling	lrWGS	PMID: 35100420
duet	SVs calling	lrWGS	PMID: 36344913
sniffles	SVs calling	lrWGS	PMID: 38168980
pbsv	SVs calling	lrWGS	https://github.com/PacificBiosciences/pbsv
SVIM	SVs calling	lrWGS	PMID: 30668829
minimap2	Alignment	srWGS, lrWGS	PMID: 29750242
winnowmap	Alignment	lrWGS	PMID: 32657365
ngmlr	Alignment	lrWGS	PMID: 29713083
lra	Alignment	lrWGS	PMID: 34153026
bwa-mem2	Alignment	srWGS	PMID: 19451168
dragmap	Alignment	srWGS	https://github.com/Illumina/DRAGMAP
bowtie2	Alignment	srWGS	PMID: 22388286
DRAGEN pipeline alignment	Alignment	srWGS	PMID: 39455800

Performances were evaluated calculating F1 score (F1), precision (P) and recall (R).

Results: For srWGS, DRAGEN yielded the best results (F1: 83% P: 90%, R: 77%). In LCR, the multi-genome outperformed hg38 (F1: 78%, P: 96%, R: 66%). Manta with minimap2 alignment showed comparable performance to DRAGEN (F1: 81%, P: 93%, R: 71%). For Nanopore lrWGS, duet was optimal at low coverages (5X - F1: 75%,P: 88%, R: 65%;10X - F1: 85%, P: 88%, R: 82%), while dysgu excelled at high coverages (20X - F1: 88%,P: 90%, R: 87%; 30X - F1: 89%, P: 90%, R: 89%). For PacBio, sniffles2 achieved the best results (F1: 90%, P: 89%, R:91%).

Conclusion: Our study showed for the first time that the choice of alignment software can drastically impact SVs calling from

srWGS. Moreover, we showed that SVs calling performances for lrWGS highly depend on the technology and the coverage level.

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P17.065.B Repurposing array data for returning clinical-grade pharmacogenetic passports to millions of biobank participants at minimal cost

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Background: Oligonucleotide genotyping arrays have genotyped millions of biobank participants worldwide. These genotyping arrays potentially offer a low-cost alternative to expensive, time-consuming single-gene assays for personalized pharmacogenetic (PGx) advice. *CYP2D6* metabolizes 25% of all drugs, but accurately genotyping this gene using oligonucleotide arrays is challenging due to a combination of single nucleotide polymorphisms, whole-gene copy number variants (CNVs) and hybrid alleles. To address this, we developed Asterix, a PGx passport pipeline with a custom *CYP2D6* CNV calling algorithm, enabling the generation of a comprehensive PGx profile for 12 genes using the Illumina Global Screening Array.

Material and Methods: *CYP2D6* variants and CNV status are modeled jointly per variant, allowing for differentiating hybrid alleles from whole-gene CNVs. For development and validation, we reanalyzed existing genetic data from the Lifelines biobank and separate validation datasets. We validated variant calling, phasing, imputation, and PGx passport accuracy using pedigree data, public reference data, and gold-standard PGx assays.

Results: We successfully validated our PGx passport pipeline. PCR-based profiling of *CYP2D6* CNV status confirmed the CNV calls from Asterix, eliminating the need for separate PCR-based detection. This opens up opportunities to return PGx profiles to millions of biobank participants that have already been genotyped worldwide. We did return 1,227 PGx passports to biobank participants via a layperson-friendly app.

Conclusion: We developed and validated a low-cost, clinical-grade PGx passport pipeline that can return comprehensive PGx profiles to biobank participants at minimal cost, ensuring effective and safe drug therapy based on their unique genetic makeup.

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P17.067.D SCALT: automatic identification of cell types from single-cell RNA sequencing data

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Background: Single cell RNA sequencing (scRNAseq) marks a methodological breakthrough for the study of the organization and function of cells and cell types. Dimensionality reduction and unsupervised clustering represent the de facto standard methods for the analysis of scRNA-seq data. These techniques however require extensive manual curation/interpretation and results are not always reproducible. Here, we present SCALT, an innovative method which introduces a paradigm-shift for the annotation of cell identities from scRNAseq data.

Material and Methods: SCALT is implemented as a collection of R and Python scripts and is available at https://github.com/danitrave/SCALT. The method was tested on a reference standard benchmark and validated by using extensive collection of expert annotated cell identities from the DISCO and HPA repositories.

Results: SCALT is a novel information-theoretic based method for the identification/classification of cell types from scRNAseq data. Key features include: automatic identification of cell type marker genes, identification and stratification of similar cell types, confidence intervals for the annotation of alternative cell type identities. Applied to two reference benchmark datasets SCALT outperformed other state of the art methods, and correctly classified with the expert curated/assigned identity 95.3% of the 5 M distinct cell in the DISCO and HPA databases. The tool can currently annotate 293 distinct operationally defined cell type identities. Custom types can also be defined.

Conclusion: SCALT introduces an innovative approach for single cell RNA sequencing data analysis, and offers a reproducible and human interpretable framework for the delineation of cell types and their annotation.

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P17.068.A Accurate detection of clinical Copy Number Variants in exomes with X chromosome reader-based Machine Learning

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Medical Sciences, University of Ferrara, Ferrara, Italy, [8]Faculty of Medicine and Cologne University Hospital, Cologne Center for Genomics, University of Cologne, Cologne, Italy, [9]Department of Information Engineering, University of Florence, Florence, Italy.

Background: Exome sequencing (ES) is now unanimously considered the first-tier diagnostic test for most genetic conditions. Although a plethora of ES-based CNV callers has been developed, each shows good performance in only a limited range of calling conditions or CNV classes and sizes. More flexibility is therefore needed for broad and robust clinical use.

Material and Methods: miXer is a light-weight machine learning tool that leverages the natural occurrence of one and two copies of the non pseudoautosomal X-chromosome in male and female exomes to discriminate genomic deletions and duplications. miXer was trained on real X-chromosome reads obtained by 6 widely used kits to capture most of the technical variability inherent to ES.

Results: Introducing a rigorous exon-level performance evaluation, we benchmarked miXer against state-of-the-art callers (ExomeDepth, DECoN, GATK-gCNV, CNVkit, EXCAVATOR2) on a comprehensive range of dbVar CNV examples synthetically embedded into the NA12878 exome, on a large ES dataset previously profiled with high-density SNP-arrays and on ES data of MLPA-validated intragenic DMD deletions. miXer globally outperformed the other callers on the synthetic benchmark, achieved higher accuracy on SNP-arrays than GATK-gCNV at the same working conditions (recall = 63%, specificity = 1) and showed sensitivity down to 2-exons for MLPA-validated events.

Conclusion: Importantly, miXer can generalize with unseen settings, requires a limited control-pool (10) and can be used either as a pre-trained model or after ad-hoc re-training on a small number (50) of samples, thus offering a reliable, resource-efficient, and highly accurate tool for clinical genomics and research applications. miXer is freely distributed as Docker package (http://github.com/ctglab/miXer).

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P17.069.B Illuminating cellular mechanisms of Mendelian and complex diseases using interpretable machine-learning

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Background: The cellular basis of many Mendelian diseases, including the causal aberration, affected cell type, and aberration mode-of-action, remains unclear, hampering disease understanding and treatment.

Material and Methods: Here, we present an extensive machine learning (ML) framework, denoted DisCell, to address each of these challenges. DisCell harnesses single-cell transcriptomic, interatomic, and functional gene features of ~22,000 protein-coding genes across 172 fetal cell types from 15 main human organs.

Results: When applied to predict aberrant genes underlying inherited retinal disorder (IRD) and autism spectrum disorder (ASD) as case studies, DisCell achieved median auROC of 0.9 and auPRC of 0.6 (expected 0.1). In a larger set of 467 diseases and 31 cell types, DisCell improved considerably the prediction of affected cell types, relative to a recently-published method that utilized preferential gene expression (F1 score of 0.33 vs 0.19). Notably, the interpretation of the resulting DisCell models provided insight into disease processes.

Conclusion: Altogether, we show that interpretable ML methods combined with rich single-cell omics data can advance the genetic and cellular understanding of monogenic and complex diseases. The DisCell framework is available online for usage by non-programmers via a web interface at https://netbio.bgu.ac.il/discell/.

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P17.070.C CADD-SV v2.0 effectively scores structural variant deleteriousness from coordinate and sequence-derived annotations

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Background: Structural variants (SVs) are large genomic alterations, with potentially significant impacts on disease. While the interpretation of individual SV effects remains challenging, CADD-SV is a comprehensive tool designed to annotate and score the deleteriousness of SVs based on diverse genomic annotations.

Methods: We present an updated machine learning model that uses an expanded training set (labels and features) and overcomes the need of separate models to score SV effects. Thereby, it supports a broad spectrum of SV types, including deletions, insertions, duplications, and inversions. Our new model combines the rich coordinate-based annotations with sequence-derived annotations extracted using SegmentNT, a DNA language model (DLM) predicting DNA elements at single-nucleotide resolution. For example, SegmentNT predicts genes, exons, introns, splice sites, promoters, enhancers, and untranslated regions from only sequence. Utilizing this state-of-the-art DLM, CADD-SV v2.0 leverages the sequence of the altered genomic regions. With the reference sequence surrounding the SV as well as its new sequence as input, it provides sequence-specific annotations and information about potentially altered molecular effects.

Results: Extensive validation of our new model demonstrated substantial improvements over CADD-SV v1.1 and other SV scoring tools in prioritizing functionally relevant SVs. CADD-SV v2.0 achieves higher accuracy and recall across SV types and enables the scoring of inversions in addition to enhanced insertion and duplication evaluation.

Conclusion: CADD-SV v2.0 provides an improved framework for annotating and prioritizing SVs, offering greater accuracy in interpreting their functional impact and aiding in understanding their roles in disease.

C.O.I. Disclosure:

O. Catona: None. **M. Kircher:** None.

P17.071.D More detail on epigenetic characteristics of enhancers and promoters

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Identification of regulatory elements is crucial for understanding gene regulatory networks. With the development of RNA-seq techniques, promoters can be easily detected based on experimentally determined transcription start sites (TSSs). Whereas,

identification of enhancers remains challenging and largely depends on chromatin profiles including chromatin accessibility and histone modifications.

In this study, we identify 10,672 putative enhancers using nascent RNA-seq profiles in the human K562 cell line. While the majority of identified enhancers exhibit classical enhancer features, a subset of enhancers located near TSSs have distinct chromatin patterns: These proximal enhancers share similar high levels of ATAC-seq as well as H3K27ac ChIP-seq with classical enhancers, but their H3K4me3 levels exceed the H3K4me1 levels, resembling promoter profiles more closely than classical enhancers.

To further explore proximal enhancers, we examine the epigenetic characteristics of identified enhancers. Because there are many CpG islands around TSSs, proximal enhancers generally have higher CpG content than distal enhancers. We find that H3K4me3 levels positively correlate and H3K4me1 levels negatively correlate with CpG content. This relationship applies to all identified enhancers and accounts for the atypical chromatin patterns observed at proximal enhancers. Interestingly, this trend is not observed at promoters, where H3K4me3 consistently dominates over H3K4me1 regardless of CpG content.

In conclusion, proximal enhancers complicate enhancer identification based on chromatin profiles, as their distinct features can reduce predictive accuracy. Future efforts will focus on developing improved strategies to accurately identify both classical and proximal enhancers.

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Y. Zhang: None. **A. Bressin:** None. **E. Altendorfer:** None. **A. Mayer:** None. **M. Vingron:** None.

P17.072.A Bayes PoPS: A Novel Bayesian Approach for Improved Polygenic Gene Prioritization in Complex Trait Genetics

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Background: Polygenic prioritization scoring (PoPS) integrates genome-wide association study (GWAS) summary statistics to identify genes associated with complex traits and diseases. It combines polygenic signals, linkage disequilibrium, and functional annotations, to assign a prioritization score to each gene. We demonstrate superior performance of a novel extension, namely Bayes PoPS.

Material and Methods: Bayes PoPS incorporates BayesR prior, eliminating the initial feature selection in PoPS. We simulated quantitative phenotypes using genotypic data from 335,000 UK Biobank participants across eight different genomic scenarios (Table), with ten independent replicates for each scenario. To assess various gene-set characteristics, we generated sets containing 20 to 100 genes, with five or twenty designated as causal (n_c). We calculated VEGAS gene-level association statistics, and derived PoPS and Bayes PoPS scores by utilising the gene-sets. The performance of each gene prioritization method was evaluated by area under the receiver operating characteristic curve (AUC).

Results: Across all simulations, Bayes PoPS consistently outperformed PoPS ($P < 2.2{\cdot}10^{-16}$). In scenarios with a higher proportion of genome-wide causal SNPs and few causal genes within gene-sets ($n_c = 5$), VEGAS outperformed both PoPS and Bayes PoPS. The power of Bayes PoPS to correctly prioritize genes significantly improved with a higher fraction of causal genes within gene-sets ($n_c = 20$).

Conclusion: Bayes PoPS demonstrated improved power to prioritize genes and robustly distinguish causal genes from polygenic background. This enhances gene discovery and interpretation in complex trait genetics, offer insights for downstream functional analyses and personalized medicine applications.

Genomic parameters	Mean(AUC) (s.d.)				
	VEGAS	PoPS		Bayes PoPS	
		$n_c = 5$	$n_c = 20$	$n_c = 20$	$n_c = 20$
Causal SNP-effects sampled from one Gaussian distribution					
$h^2 = 0.3$, $\pi = 0.001$	0.90 (0.01)	0.88 (0.02)	0.99 (0.01)	0.92 (0.01)	0.99 (0.01)
$h^2 = 0.3$, $\pi = 0.01$	0.76 (0.01)	0.54 (0.01)	0.76 (0.01)	0.59 (0.01)	0.79 (0.01)
$h^2 = 0.1$, $\pi = 0.001$	0.86 (0.02)	0.87 (0.01)	0.99 (0.01)	0.91 (0.01)	0.99 (0.01)
$h^2 = 0.1$, $\pi = 0.01$	0.67 (0.01)	0.54 (0.01)	0.72 (0.01)	0.57 (0.01)	0.75 (0.01)
Causal SNP-effects sampled from three different Gaussian distributions					
$h^2 = 0.3$, $\pi = 0.001$	0.88 (0.02)	0.87 (0.02)	0.99 (0.01)	0.92 (0.01)	0.99 (0.01)
$h^2 = 0.3$, $\pi = 0.01$	0.73 (0.01)	0.54 (0.01)	0.76 (0.01)	0.58 (0.01)	0.78 (0.01)
$h^2 = 0.1$, $\pi = 0.001$	0.82 (0.02)	0.85 (0.03)	0.99 (0.01)	0.88 (0.02)	0.99 (0.01)
$h^2 = 0.1$, $\pi = 0.01$	0.65 (0.01)	0.54 (0.01)	0.70 (0.01)	0.56 (0.01)	0.74 (0.02)

h^2: SNP-heritability, π: proportion of causal SNPs.

C.O.I. Disclosure:

P.D. Rohde: None. **P. Sørensen:** None.

P17.073.B VarChat: leveraging generative AI to enhance genomic literature retrieval and summarization

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Background: The growing volume of genomic literature presents significant challenges for the efficient retrieval and synthesis of variant-specific information, which is essential for clinical and research applications. To address these challenges, we introduce VarChat, an innovative generative AI-based platform that leverages Large Language Models (LLMs) to optimize the retrieval and summarization of up-to-date literature, directly referencing genomic variants of interest.

Materials and Methods: VarChat integrates multiple literature databases within a hybrid Retrieval-Augmented Generation framework, combining sparse and dense retrieval approaches. It synthesizes fragmented findings into concise, scientifically accurate summaries while providing access to high-confidence references. Additionally, the platform features a guided chat interface, enabling users to explore specific functional and clinical implications of genomic variants. A LLM-based Named Entity Recognition (NER) module has been developed to enhance literature retrieval and expand coverage to include new variant types, such as CNVs and pharmacogenomic alleles.

Results: VarChat is freely accessible at varchat.engenome.com, natively integrated in eVai (evai.engenome.com), and recently into the UCSC Genome Browser. Preliminary results on a ClinVar-based

benchmark demonstrated that the LLM-Based NER module used for literature retrieval achieved a sensitivity of 72% in identifying variants with standard nomenclature, increasing to 91% when accounting for correct identification under non-standard nomenclature (e.g., unofficial gene symbols). Missed variants were located in supplementary materials, not currently preprocessed by the module.

Conclusions: By combining advanced literature preprocessing with hybrid retrieval techniques, VarChat accelerates the interpretation of genomic variants, providing critical support to the genomic community in advancing clinical decision-making.

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P17.074.C Single cell transcriptome analysis and inference by machine learning on prostate cancer fusion biopsy specimens identifies cancer-associated cells with diagnostic gene expression signatures

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Background: Prostate cancer (PC) is the second most common malignancy worldwide and the leading cancer in men. The PC tumour microenvironment conditions precise patient stratification and treatment efficacy. Single-cell RNA sequencing (scRNA-seq) enables detailed characterization of this complexity, while transperineal fusion-guided biopsy enhances diagnostic precision due to its higher specificity. However, studies using scRNA-seq from fusion-guided biopsy samples at diagnosis are limited.

Material and Methods: We analyzed the tumor microenvironment of 31 fusion-guided biopsies from 24 newly diagnosed PC patients, categorized into high (19 samples) and low (21 samples) tumorigenicity groups. scRNA-seq was performed to identify cancer cells enriched with PC-associated signatures. Differential abundance analyses using k-Nearest Neighbourhood clustering and pseudobulk gene expression data were used to develop machine learning models, including Elastic Net, validated via Leave-One-Out Cross Validation (LOOCV) and Bootstrap.

Results: scRNA-seq analysis revealed PC cell subpopulation such as PC cells and cancer-associated fibroblasts (CAFs). Differential abundance analysis identified high tumorgenicity-enriched cells (SpatialFDR < 0.01) characterized by overexpression of a subset of 52 genes such as *PCA3*, *SCHLAP1*, *CRISP3*, and *ERG*. Elastic Net models with LOOCV from the pseudobulk expression of these 52 genes showed robust performance (Accuracy, F1-score, G-mean, and AUC: 0.61–0.71). SHAP analysis identified *PCA3*, *TNFSF10*, *CPNE4*, and the novel *ENSG00000289332* as key discriminators of high vs. low tumorigenicity.

Conclusion: This study highlights the novel use of scRNA-seq in fusion-guided biopsy samples for PC diagnosis, with identification of PC and CAF cells and potential for new biomarker discovery, including the novel long non-coding RNA *ENSG00000289332*.

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P17.075.D Weight loss maintenance metabolome and microbiome predictors

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Background: Obesity poses many health-related and societal problems—successful weight loss maintenance (WL & WLM) remains challenging. The Finnish ELIPA food intervention study for WLM targeted this challenge while measuring gut microbiome and metabolome changes. We evaluated these omics factors contribution to WLM.

Material and Methods: ELIPA lasted 33 weeks, with sampling made at baseline before weight loss (stage1-week0), after WL/pre (stage2-week9), during (stage3-week21) and after weight maintenance (stage4-week33). Absolute weight change was modelled using random forest regression with 5-fold cross-validation. Metabolomic feature velocities (the rate of changes in abundance over WL, stages 1–2) were selected from 90 features via LASSO regression, while gut microbiome velocities (derived from enterotype clusters) were selected using linear regression before testing their relationships with weight change. Tested models were: two in full cohort (n = 82) with age/sex+/− metabolomics; two in microbiome subset (n = 32) with metabolomics+/-enterotype dynamics.

Results: In full cohort, glycine betaine velocity (variable importance, VI = 100%) was the strongest predictor of weight change. The microbiome enterotype velocity Cluster2 including *Faecalibacterium prausnitzii* contributed significantly (VI = 74%) when added to WLM model. In microbiome subset, stearamide (VI = 60.1%) and glycosidic compound MW488.3363 (VI = 100%) were important predictors combined with enterotype Cluster2 velocity (VI = 54%). The highest predictive accuracy was achieved for model including microbiome subset.

Conclusion: Dynamic changes in glycine betaine and stearamide metabolites are important for WLM with microbiome data enhancing model performance. These results highlight opportunity for personalized WLM strategies through targeted metabolite and microbial adaptation. Further validation in larger studies is needed to advance these biomarkers into clinical interventions.

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P17.076.A Antiepileptic drugs effects on embryonic development: a transcriptome meta-analysis of in vitro studies and assessment of exposed newborns

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Background: Antiepileptic drugs might present a risk for embryonic development, potentially causing craniofacial anomalies, congenital heart defects (CHD), and neural tube defects (NTD). However, the knowledge of antiepileptic-induced molecular disturbances in the embryo is scarce. Hence, this study aimed to evaluate genes differentially expressed (DEG) in human embryonic cells (hEC) and organoids exposed to antiepileptics, to compare these results to the DEG of newborns exposed to antiepileptics prenatally.

Material and Methods: Datasets of hEC or organoids exposed to antiepileptics were obtained in the GEO repository. Transcriptomes selected were processed in R language, evaluating DEG. A transcriptome meta-analysis was performed using the Fisher-P method. Physical examination of five newborns prenatally exposed to antiepileptics was performed and saliva samples were collected for RNA-seq performance.

Results: hEC transcriptome meta-analysis comprised 17 datasets of valproic acid (VPA) and six datasets of carbamazepine exposure; 317 DEG were identified. A second meta-analysis, of four organoids exposed to VPA, presented 411 DEG. Fifty-three DEG were common between both analyses: the homeobox *DLX4*, involved in craniofacial development; *TBX18*, a cardiovascular development transcription factor; and *NPFFR2*, a neurodevelopment gene. The RNA-seqs of five newborns, two exposed to VPA and three to carbamazepine (first trimester), are under evaluation. Two newborns present CHD, one exposed to VPA and another to carbamazepine; a third newborn, also exposed to carbamazepine, presents NTD.

Conclusion: Transcriptome meta-analysis based on in vitro assays can provide insights into genes potentially implicated in antiepileptics-induced embryopathies. This preliminary result will be confirmed by accessing the transcriptome of prenatally-exposed newborns.

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T.W. Kowalski: None. **J.V. Foletto:** None. **L. Schuler-Faccini:** None. **M.T. Sanseverino:** None. **F.S. Vianna:** None.

P17.077.B A deep learning based web application for chromosomal abnormality detection using microscopic images

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Background: Chromosomal abnormality, a major genetic disorder causing infertility and abortion, can be detected using traditional, time-consuming karyotype analysis methods, but advancements in image processing and deep learning offer potential automation.

Material and Methods: This study used microscopic chromosome images from the Gel Cell Laboratory to train a deep learning model. The initial model had suboptimal performance due to class imbalance. To address this, data balancing techniques were applied, and a refined dataset of 2,832 images was selected for training and optimization. Data augmentation techniques were applied to enhance the dataset. A Convolutional Neural Network (CNN) architecture and pretrained models were used, and performance was assessed using key metrics like accuracy, precision, recall, and F1-score. Ultimately, we designed a web application for evaluating model's performance on unseen local data.

Results: The CNN-based model demonstrated exceptional accuracy in identifying chromosomal anomalies, with 87% accuracy on the validation dataset and 85.03% accuracy on the test. It achieved precision of 83% and recall of 82%, demonstrating a balance between identifying positive cases and minimizing false predictions. Data augmentation improved performance, particularly in handling low-quality images. The model has been tested on local data through designed web application and achieved satisfying result.

Conclusion: The study demonstrates that deep learning, specifically CNNs, can effectively detect chromosomal abnormalities from microscopic images, providing a reliable tool for genetic laboratories for rapid and precise disease diagnosis, with future advancements exploring transfer learning.

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M. Zare: None. **F. Modabber:** None. **S. Movahed:** None. **N. Ghasemi:** None.

P17.078.C Comprehensive in silico functional analyses of *HR* Gene pathogenic variants against Alopecia areata

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Background: Alopecia areata is an autoimmune disorder characterized by abnormal hair loss, affecting approximately 2% of the global population. Research has linked the *HR* gene, which encodes the HR Lysine Demethylase and Nuclear Receptor Corepressor, located on chromosome 8. This study investigates the role of pathogenic variants in *HR* Gene through in silico methods to analyse their potential role in the pathogenesis of Alopecia areata.

Materials & Methods: A comprehensive analysis of these 297 single nucleotide polymorphisms (SNPs) in the *HR* Gene was performed, Identifying 30 variants as deleterious. Among these, four key SNPs, especially R1097H, R1099L, G1114R, and V1136D, exhibited the most substantial structural and functional effects on the protein, as determined by conserved and domain region analyses. The 3D structural predictions of the HR protein were generated using AlphaFold, and the results were further validated with I-TASSER. Molecular docking studies were then conducted to evaluate interactions with gene specific small molecules.

Results: N-oxalylglycine exhibited the lowest binding energy of −5.2 kcal/mol and formed strong interactions within 4Å of the following residues: Ala980, Leu981, Pro983, Leu984, Asp1012, Leu1013, Val1014, Ser1015, Leu1046, Gly1047, Ser1048, Gln1049, Arg1114, C1153, and Pro1157. Notably, N-oxalylglycine interacted with the significant SNP residue G1114R, highlighting its potential as a therapeutic candidate.

Conclusion: This study highlights the potential pathogenic role of specific *HR* gene variants in alopecia areata and identifies N-oxalylglycine as a promising molecule for further investigation. These findings contribute to the understanding of the molecular basis of Alopecia and opens approaches for targeted therapeutics.

C.O.I. Disclosure:

K. Bonthala: None. **C. Kb:** None. **M. Manne:** None.

P17.079.D Ancestry-specific database for cross-tissue Gene Expression Models: AGEMdb

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Background: Transcriptome-wide association studies (TWAS) link genetically regulated gene expression to traits or diseases. TWAS uses eQTL to predict gene expression and tests the association between the predicted expression and a trait. TWAS success relies on the accuracy of gene expression imputation models, which are trained using both genotype and gene expression data from the same samples. However, available resources, like the Genotype Tissue Expression (GTEx) Project, mainly focus on individuals of European ancestry, limiting prediction accuracy for other ancestry groups. To address this, we introduce the 'AGEMdb' (Ancestry-specific Gene Expression Models database), which includes gene expression models across 49 tissues for both European ancestry and African-American individuals in GTEx.

Material and Methods: Genotype and gene expression data were obtained from GTEx. After genotype quality-control, data from 689 European ancestry individuals and 111 African-American individual were retained. Gene expression was adjusted for confounding factors, and cross-tissue UTMOST models were trained separately for each ancestry group. These models were then used as input to the database.

Results: 'AGEMdb' is a web-based database hosting pre-trained gene expression imputation models in GTEx. Users can browse, search, and download models with flexible filtering by tissue type and gene. These models enable gene-trait association analysis in TWAS (UTMOST and similar tools) by predicting tissue-specific gene expression from genomic data. The inclusion of African-American models reduces European ancestry bias in human genetic/omic resources, enhancing inclusivity.

Conclusion: The 'AGEMdb' database is a valuable resource for ancestry-specific TWAS analysis, enhancing multi-tissue gene expression prediction accuracy across diverse populations.

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P17.080.A Integration of federated learning to study of pathologies with intellectual disabilities within the European BETTER Consortium

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Background: The study of pediatric rare diseases associated with intellectual disability, such as syndromic neurodevelopmental disorders (sNDD) and inborn errors of metabolism (IEM), faces significant challenges due to the limited number of cases available. This limitation prevents the generation of large patient cohorts necessary for the accomplishment of statistically robust studies and generation of new knowledge for better diagnosis and prognosis.

Material and Methods: Currently, within the European BETTER Consortium, we have selected 309 patients with sNDD, encompassing chromatinopathies and RASopathies. From these patients, we have obtained genomic data through next-generation sequencing (NGS), combined with phenotypic traits (HPOs) and functional biological data from early or late-diagnosed patients. These data shall be integrated in accordance with the FAIR principles into a federated platform, PADME (https://padme-analytics.de), which is capable of securely running distributed analyses at multiple European institutions and combining their data.

Results: A first prototype model of a federated analysis has been developed to work with a preliminary subset of data, being able to work with anonymized and interoperable datasets. This model of federated learning seeks to improve the specificity of the results obtained within each cohort, enabling work with multiple institutions that have real-world data, while ensuring GDPR compliance and increasing the robustness of the analyses.

Conclusion: This innovative approach, that integrates real-world and federated data, aims to delineate the causes of intellectual disability, and enhance the diagnosis and genotype-to-phenotype prediction of sNDD and IEM through the identification of novel genetic biomarkers, genetic modifiers, and/or disrupted signaling pathways.

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P17.081.B GenDaB - a web database for human genes

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Background: To assess a gene's involvement in a specific disease, researchers often have to comb through different websites to retrieve all information they need. Navigating through multiple

data sources and extracting only the required information is very labor-intensive and time consuming. We aim to streamline this process by providing a comprehensive and customizable one-stop-shop database for human genes.

Material and Methods: We have developed GenDaB, a user-friendly web-based database for human genes which allows researchers to retrieve the specific information they are interested in. It integrates data from various fields, including phenotypic data associated with a gene, protein function, gene and protein expression data, and protein-protein interactions. Users can query GenDaB with a list of genes or genomic regions. They can also filter genes for their specific properties, such as expression in a target tissue or known correlation with clinical signs.

GenDaB includes an API for easy integration in other applications or pipelines.

Results: GenDaB offers a user-friendly and comprehensive resource for researchers investigating human genes and their involvement in known diseases. The extensive search, filter and display options allow users to customize their queries based on their individual needs and retrieve only the information relevant to their research interest.

Conclusion: The GenDaB database is freely accessible at https://www.genecascade.org/gendab. It provides researchers with a powerful tool to gain comprehensive insights into human genes and their potential roles in diseases.

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P17.082.C FinemappInG And pleiotRopy sOlver (FIGARO) pipeline for disentangling pleiotropy of large datasets

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Background: One of the main goals of genetic epidemiology is to identify variants influencing complex traits and understand how they exert their effects through biological layers. This helps reconstruct the causal relationships between traits. Given the abundance of genetic association studies available today, new tools are needed to efficiently process and summarise these relationships.

Material and Methods: We developed a statistical framework for untangling any type of genetic association summary statistics which performs: GWAS summary statistics harmonisation, identification of associated genomic regions through an ad hoc positional algorithm, fine-mapping of identified loci with SuSiE, pairwise colocalisation analysis, identification of coregulated association signals and joint fine-mapping by leveraging the results of multiple, colocalising traits to increase its precision. The framework relies on a combination of Nextflow pipeline and efficient storage of minimal data in AnnData objects to reduce computational and storage burden.

Results: We applied our approach to 10,641 eGenes over 28 cell types, resulting in 47,480 cis-eQTL tests carried out within the Cardinal study. After fine-mapping, the 79,416 credible sets (CS) obtained were stored in an AnnData object, reducing the disk storage by ~788 times. Colocalization was performed on pairs of CS sharing at least one SNP, resulting in 273,404 pairwise colocalization tests. CS were then grouped in 18,910 cis-regulated modules where the underlying causal SNP was shared across all.

Conclusion: Our pipeline provides a scalable, flexible, and memory-efficient solution for fine-mapping and colocalisation analysis of large-scale association data, helping uncover biologically relevant co-regulation mechanisms across diverse phenotypes.

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P17.083.D Multiomic profiling of antibiotic response: Ampicillin, Aztreonam, Gentamicin and Cefepime's effect on multidrug-resistant *E. coli* strains

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Background: The misuse of antimicrobials has led to the rapid spread of antimicrobial resistance (AMR) in microorganisms, presenting a significant global health threat to effective infection treatments. Understanding how microorganisms respond to antibiotics is essential for developing new therapeutic strategies and advancing drug discovery.

Material and Methods: We investigated the proteomic, metabolomic and genomic profiles of three *Escherichia coli* (*E. coli*) strains with varying antibiotic resistance profiles, in comparison to a reference *E. coli* K12 strain. The focus was on strains exhibiting multidrug resistance to ampicillin, aztreonam, gentamicin, and cefepime, highlighting the role of active efflux pumps in antibiotic resistance mechanisms. We used UPLC and timsTOF-MS/MS to analyze metabolomic and proteomic profiles.

Results: Through integrated proteomic and metabolomic analyses, 450 proteins and 350 metabolites associated with resistance in the selected strains were identified. These findings indicate potential biomarkers that contribute to the bacterial survival under antibiotic stress. Notably, we observed differential protein expression linked to key metabolic shifts that facilitate resistance. These include changes in pathways related to cell wall biosynthesis, energy production, and stress response, which are critical for maintaining bacterial viability in the presence of antibiotics. Additional genome sequencing determined whether the changes in proteins originated from antibiotic treatment or from mutations in the genomes of the three *E. coli* strains.

Conclusion: Our findings underscore the potential of multi-omics approaches in advancing the understanding of antibiotic resistance and highlight the proteins and metabolites that may serve as targets for the development of novel antibiotics or resistance-modifying agents.

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P17.084.A Transcript level quantification and splice junction detection in single-cell short-read expression data

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Background: The analysis of splice junctions (SJ) and transcript expression at the single cell level informs understanding of cell-type specific patterns of gene regulation, giving new insights into cell biology, human traits, and disease mechanisms where alternative splicing is modified despite overall stable gene expression. However, transcript quantification and SJ detection are poorly explored in 10X Genomics scRNA-seq data.

Material and Methods: We compared computational methods for quantifying isoforms from 10X Genomics scRNA data, namely STARSolo, salmon-alevin, and kallisto-bustools. Using publicly available data and in-house generated data from 10X 3' and 5' libraries and relying on long-read single-cell data as ground truth, we compared the performance in quantifying isoform expression, transcript ranking, and transcript ratios within genes. We performed SJ analysis using LeafCutter (as described in Tian et al., Nature Genetics, 2024) and transcript quantification using the best-performing method on pseudo-bulk data in a cohort of ~5000 Europeanindividuals from the UK Biobank (CARDINAL project).

Results: Results show that scRNA data can be used to compare SJ usage and transcript quantification at the pseudo bulk cell-type level, and we propose a strategy to select genes for reliable transcript quantification. Preliminary data on the CARDINAL dataset demonstrate how our workflow can generate isoform and SJ quantification suitable for investigating the association with phenotype and genotypes.

Conclusion: We present an effective strategy for analyzing transcript-level expression in scRNA-seq datasets generated using 10X technology, and we show how this data can be used to gain new insights into cell-type specific transcript usage.

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P17.085.B Comparative bioinformatics analysis of the transcriptome in High-Grade Serous Ovarian Cancer (HGSOC)

Beatrice Melli[1,2], *Vincenza Ylenia Cusenza*[2], *Valentina Vaccari*[3], *Sandra Martinelli*[2], *Eleonora Zanetti*[4], *Andrea Palicelli*[4], *Vincenzo Dario Mandato*[1], *Lorenzo Aguzzoli*[1], *Davide Nicoli*[2], *Roberta Zuntini*[3]

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Background: High-grade serous ovarian cancer (HGSOC) accounts for 80% of ovarian cancer deaths. The heterogeneity of HGSOC leads to therapeutic resistance and poor outcomes. Exploration of differentially expressed genes (DEGs), performing pathway enrichment analysis (PEA), and analyzing protein-protein interaction (PPI) network through transcriptome analysis could identify new molecular mechanisms involved in this tumor and improve the knowledge about the heterogeneity of HGSOC.

Material and Methods: Preliminary Transcriptomic analyses of 8 samples of HGSOC were performed on RStudio (v4.3.2.) using the Bioconductor packages "EDASeq" for quality control; "edgeR", "DeSeq2", "limma" for DEGs analysis; "ReactomePA", "clusterProfiler" for PEA and "STRINGdb" for PPI analysis.

Results: The integration of results from bioinformatics analyses performed using the "edgeR", "DeSeq2" and "limma" packages showed 9,674 upregulated and 7,536 downregulated common DEGs on 20,812 genes. Validation of DEGs was conducted through real-time PCR, focusing on the top 50 commonly selected genes based on absolute logFC and p-value. These preliminary findings confirm the upregulation of the *PAX8* gene and highlight the involvement of the PI3K-Akt signaling pathway, as supported by the literature. The next step involves the comparative integration of additional bioinformatics tools to explore PEA and PPI network analyses, including the difference in results between analysis.

Conclusion: Comparing different Bioinformatics tools for the analysis of transcriptome could help to understand the molecular alteration involved in the pathogenicity and have a confirmation of obtained results. Furthermore, in-depth analysis can help to highlight different results to find hidden mechanisms involved in the prognosis of the tumor.

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P17.086.C Automated extraction of HPO ids from clinical notes using Large Language Models

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Background: Given the vast number of variants that need to be interpreted in Whole Exome or Whole Genome analyses, prioritizing them based on the observed phenotype is essential. The use of standardized phenotypic ontology terms (HPO codes) plays a crucial role in identifying diagnostic variants. However, the upstream collection of HPO terms remains complex, limiting their practical use in clinical settings.

Material and Methods: Automatic extraction of HPO ids from raw clinical notes relies on an agentic Artificial Intelligence called PhenX. PhenX uses a Large Language Model to identify patient symptoms, then performs suggessive tasks to match them with Terms from the Human Phenotype Ontology and their IDs. The agent takes also cares of patient anonymization and prevents hallucinations.

Results: We assessed the quantity and quality of HPO IDs extracted by the Agentic AI in a cohort of nephrology patients. Each patient had clinician-validated HPO terms derived from consultation notes. For patients with a confirmed diagnosis, we analyzed the impact of extracted phenotypes on diagnostic gene rankings using PhenoGenius, a gene-phenotype matching tool. We evaluated three extraction protocols: fully expert-curated, semi-automated, and fully automated."

Conclusion: We present and evaluate PhenX, and Agentic AI for automatic phenotype extraction. While the rankings of diagnostic gene remain lower with expert curated Phenotypes, the agentic AI offers an efficient alternative to the time consumming task of extacting phenotypes from clinical notes.

C.O.I. Disclosure:

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P17.087.D Mapping Complex HGVS Variant Descriptions Across Genetic References

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Background: The Human Genome Variation Society (HGVS) provides a set of recommendations for the accurate and unambiguous description of genetic variants. While existing methods attempt to normalize HGVS variant descriptions by independently manipulating them into a canonical form, they are generally effective only for small variants. Advancements in genomic technologies have led to more frequent recording of complex variant combinations and facilitated the development of improved and competing reference genomes (GRCh38 and T2T-CHM13). These developments drive the need for advanced solutions capable of also accurately mapping variants across references.

Material and Methods: We introduce methods for generating standardized HGVS descriptions for complex variants and mapping them between (related) reference sequences. A deterministic algorithm extracts canonical variants by comparing a reference sequence to an observed sequence. We apply this extraction process for variants of each molecular type (DNA, RNA and protein) which results in independent variant descriptions in each domain. By extracting the observed sequence against another reference, the original HGVS description is mapped while incorporating reference differences rather than merely lifting over variants. An additional extraction step identifies variants introduced by reference sequence differences, allowing them to be subsequently filtered out.

Results: Our method enables the seamless transfer of variant descriptions between different genome builds and molecular domains, while accounting for sequence differences. Furthermore, the method supports inter-species variant conversions, expanding its applicability across diverse genomic contexts.

Conclusion: These methods are integrated into the Mutalyzer 3 tool suite, available as a web application (https://mutalyzer.nl/) and a Python package (https://github.com/mutalyzer/mutalyzer).

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P17.088.A Incorporation of clinical and molecular variant properties improves classification and calibration of in silico pathogenicity prediction tools

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Background: In silico pathogenicity prediction tools are essential for determining the potential pathogenicity of genetic variants and their performance can vary depending on molecular and clinical contexts. In this study we evaluated the performance variability of commonly used tools under different conditions and recalibrated score thresholds to better reflect evidence of pathogenicity.

Materials and Methods: ClinVar variants were analyzed using 25 tools. Tools were evaluated based on discriminatory performance on several variant subsets. For each subset, Bayesian methods were employed to recalibrate score thresholds corresponding to the levels of evidence defined by the American College of Medical Genetics (ACMG).

Results: The performance of tools varied significantly across different subsets. Tools exhibited reduced accuracy for variants with higher allele frequencies and lower discriminatory performance for variants located in regions with high conservation. Variants affecting autosomal recessive (AR) and X-linked (XL) genes were more accurately classified compared to those affecting autosomal dominant (AD) genes by most tools. Using simulated variant subsets the recalibrated thresholds consistently showed higher odds of correctly estimating pathogenicity (OR = 3.78 [1.74, 8.58]; $p < 0.001$). Compared to the genome-wide thresholds, the recalibrated method produced significantly higher scores for known pathogenic/likely-pathogenic (P/LP) variants and lower scores for benign/likely-benign (B/LB). This improved discriminatory performance was particularly notable in variants found in low-conservation regions and AR genes.

Conclusion: in silico pathogenicity prediction tools exhibit context-dependent performance variability. Score threshold recalibration extends the range of evidence and improves overall pathogenicity probability estimation and classification.

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P17.090.C Enabling clinical translation of high-throughput functional assay data with MaveDB

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Background: A central problem in precision medicine is understanding the effects of individual DNA variants. Measuring their activity in the lab provides valuable evidence for clinical interpretation but can be both time- and resource-intensive. Multiplexed assays of variant effect (MAVEs) are a family of experimental techniques that allow researchers to measure many thousands of variants in a gene or other functional element in parallel, generating a large volume of high-quality functional evidence. Our goal is to increase the availability and discoverability of MAVE data for researchers and clinicians alike.

Material and Methods: We developed MaveDB, an open-source community-oriented database for MAVEs and other types of functional assays including those based on low- or medium-throughput approaches. MaveDB implements a user-friendly model for uploading and searching datasets, as well as a clinician-focused interface that provides detailed information and visualisations for individual variants. We are also driving the

creation and adoption of international standards for functional assay data.

Results: MaveDB has been embraced by the MAVE community as the database of record and already contains 2000 datasets comprising over 7 million individual variant-effect measurements. This includes MAVE data for several genes on the ACMG secondary findings list such as *BRCA1*, *CALM1/2/3*, *KCNQ1*, *MSH2*, *PTEN*, *SCN5A* and *TP53*. Availability of data standards has enabled integration of MaveDB data by diverse partner resources.

Conclusion: As more functional data continues to be generated, increasing availability and promoting standardisation will help get this important evidence into the hands of clinicians quickly to improve patient outcomes.

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P17.091.D A DNA methylation-based diagnostic algorithm for rheumatoid arthritis

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Background: Rheumatoid arthritis (RA), particularly seronegative disease, is difficult to diagnose early, which can delay treatment initiation. This study aims to develop a binary DNA methylation (DNAm)-based algorithm to diagnose RA.

Material and Methods: Three datasets (discovery, training, holdout) were constructed from DNAm profiles from 1,366 persons (treatment-naïve RA, other inflammatory/autoimmune diseases, healthy individuals). DNAm features that differentiate RA from other inflammatory/autoimmune diseases and healthy individuals were identified using the discovery set. Our classification algorithm was developed using machine learning techniques in the training set. Its diagnostic performance, with and without serological status, was evaluated in the holdout set containing RA cases (15 seropositive, 6 seronegative) and controls (14 other arthritides,11 healthy individuals).

Results: Our algorithm included 391 DNAm features (CpGs). Combined with serological status, it classified RA from controls in the holdout set with the following performance: sensitivity 0.90 [95% CI: 0.70–0.99], specificity 0.88 [95% CI: 0.69–0.97], and AUC 0.96 [95% CI: 0.91–1.00]. Its performance in classifying patients with seronegative RA versus those with other arthritides was: sensitivity 0.83 [95% CI: 0.36–1.00], specificity 0.79 [95% CI: 0.49–0.95], and AUC 0.81 [95% CI: 0.61–1.00].

Conclusion: The present DNAm-based classification algorithm may be clinically useful for the early diagnosis of RA, especially in seronegative patients.

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P17.092.A A compendium of uniformly processed human single-cell gene expression quantitative trait loci

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Background: Molecular mechanisms underlying human complex traits and diseases remain poorly understood. While large-scale gene expression quantitative trait locus (eQTL) studies from bulk tissues have had some success in interpreting genetic associations with complex traits, bulk analysis can hide important cell-type-specific regulatory effects. Although single-cell eQTL studies are now starting to emerge, different labs use a heterogeneous set of approaches for data processing and cell type annotation, complicating the integration and comparison of results from these studies.

Material and Methods: Building on the success of the eQTL Catalogue (https://www.ebi.ac.uk/eqtl/), we have compiled a set of open source workflows for uniformly processing single-cell eQTL datasets. First, we use the nf-core/scrnaseq workflow to re-quantify gene expression from scRNA-seq data. We also developed Nextflow workflows for single-cell quality control, demultiplexing, and doublet detection using tools from the Demuxafy package. We also automatically annotate cell types with the CellTypist software package. Finally, we use the existing eQTL Catalogue bulk workflow to perform genotype imputation, association testing, and statistical fine-mapping.

Results: We have finished processing data from the first six single-cell eQTL studies and have made these results publicly available on the eQTL Catalogue FTP server and our interactive browser (https://elixir.ut.ee/eqtl/). We are currently working on the uniform processing of the next five datasets from various tissues. We have also publicly released all of our workflows.

Conclusion: We believe that the single-cell eQTL Catalogue will become a valuable novel resource for GWAS interpretation and drug target prioritisation.

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P17.094.C SnakeSplice: A seamless workflow for splice variant discovery and visualization in RNA-Seq data

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Background: Correct splicing is essential for protein expression. Thus, defects in this process are associated with numerous

diseases. RNA sequencing (RNA-Seq) can be used to detect variants, and to study mRNA expression and isoform abundance. Yet, bridging the gap between complex data analysis and intuitive result presentation remains challenging.

Material and Methods: SnakeSplice is a comprehensive RNA-Seq analysis pipeline with five modules: (1) quality control, preprocessing, alignment, (2) gene fusion detection, (3) transcript quantification, differential expression, gene set enrichment analysis, (4) splicing pattern assessment and (5) variant detection. Additionally, SnakeSplice introduces PJD-tool, a novel method for identifying hitherto unknown "private" splice junctions. To evaluate its performance, we analyzed three positive control datasets (5/3/1 cases, 20 controls) with known splicing defects in BUD13, SUPT7L, and MRPL42, respectively, as well as 71 additional transcriptomic samples for exploratory analysis.

Results: SnakeSplice efficiently processes large-scale datasets by leveraging parallel computing and High-Performance Computing (HPC) resources. To enhance usability, SnakeSplice generates interactive HTML reports, making results easily accessible to broader research and clinical teams. The PJD-tool significantly improves the identification of novel splice sites, contributing to a deeper understanding of alternative splicing. In our validation, SnakeSplice correctly prioritized the known pathogenic splicing defects, ranking BUD13 and MRPL42 as top candidates and SUPT7L among the top 10 genes for further investigation.

Conclusion: SnakeSplice is an open-source, scalable workflow optimized for parallel computing environments. It provides an end-to-end solution for gene expression and splicing analysis, including detection of novel splice sites and generation of interactive HTML reports.

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P17.095.D AION: an AI-driven dual-model variant interpretation framework for diagnostics and discovery

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Background: As a regulatory-compliant variant interpretation platform, AION undergoes routine evaluation guided by clinical performance metrics. Balancing sensitivity and specificity is particularly challenging in genetic diagnostics, as capturing the long tail of pathogenic variants can increase false positives. Therefore, clinical performance must be tailored to the application: high specificity for diagnostics and high sensitivity for research.

Methods: To address these distinct needs, we have developed a dual-model variant prioritization framework in AION: a high-stringency approach, which prioritizes specificity for screening and diagnostics, and a low-stringency approach, which prioritizes sensitivity for research. We evaluated AION v3.14.0.0 across three datasets: 1) a synthetic dataset with disease-causing variants ($n = 1089$), 2) real-world clinical cases with diverse genetic conditions ($n = 125$) and 3) a negative control dataset where healthy controls were annotated with Mendelian disease phenotypes ($n = 100$). Sensitivity, ranking and specificity were assessed to measure clinical performance.

Results: The low-stringency model showed higher sensitivity (synthetic: 94.4% vs 89%; clinical: 85.2% vs 74.6%), but prioritized more candidate diagnoses (synthetic: 11.1 vs 1.12; clinical: 18.2 vs 2.2). Conversely, the high-stringency model accurately identified 41% of the negative dataset as having no pathogenic variants, requiring on average a review of only 1.17 results per case, underscoring its utility in diagnostics.

Conclusions: AION's dual-model framework provides a flexible, robust balance of sensitivity and specificity, leading to practical benefits like faster case review, improved detection of negative cases and higher case resolution rates. This ultimately enables more confident conclusions in variant interpretation, enhancing both diagnostic accuracy and speed and research efficiency.

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P17.097.B A Framework to Maximise Genetic Diversity in Genome-Wide Association Study Meta-Analyses

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There have been recent efforts by the human genetics research community to increase the genetic diversity of participants contributing to genome-wide association studies (GWAS). The standard approach is to first assign participants to an "ancestry group" and then aggregate results across ancestry-specific GWAS through multi-ancestry meta-analysis. However, participants are typically assigned "continental" labels that may not reflect personal views of ethnicity/race or fully represent genetic diversity. Furthermore, some admixed participants are excluded because they cannot be assigned to a single ancestry group. Here, we present a novel pipeline for multi-ancestry meta-analysis that employs a continuous and multi-dimensional representation of ancestry by first projecting all participants onto genetic principal components (PCs) derived from the Human Genetic Diversity Project. Association analyses are then conducted for all participants in a mixed model with adjustment for PCs as covariates. Testing for interaction with PCs enables assessment of ancestry-correlated heterogeneity. We applied the new pipeline to meta-analysis of GWAS of type 2 diabetes in up to 55,090 cases and 503,503 controls of diverse ancestry. Despite increasing the genetic diversity of participants included in the meta-analysis, we demonstrate that association summary statistics were not more inflated for the new pipeline than the standard approach (genomic control lambda 1.159 for both). The new pipeline identifies 170 loci at genome-wide significance ($P < 5 \times 10^{-8}$), compared with 159 for the standard approach. The new pipeline increases sample size, and the diversity of participants included in multi-ancestry GWAS meta-analysis, offering enhanced power for discovery of loci that are relevant across global populations.

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C. Yap: None. **A.P. Morris:** None.

P17.099.D HLA-DRBindPred: An Explainable Machine Learning Identifier for HLA-DR4 Binding peptides

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Background: HLA-DRB1*04:01 is associated with several autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, diabetes, as well as with COVID-19. The identification of peptides binding to this allele is crucial for the development of immunotherapies and vaccines. Existing methods for predicting HLA-DRB1*04:01 binders have low accuracy, reducing their

reliability in identifying binders for immunological research and clinical applications.

Methods: In this study, we propose HLA-DR4BindPred, a novel machine learning-based predictor for identifying HLA-DRB1*04:01 binding peptides using 100,0000 peptide sequences. It utilizes a range of feature encoding schemes, including composite protein sequence representation (CPSR), extended pseudo amino acid composition (ExPseAAC), and dipeptide deviation from expected mean (DDE) to capture essential information from peptide sequences. Finally, random forest-based machine learning model was developed to identify HLA-DR4 binding peptides. Novel peptide motifs were also identified using MEME Suite.

Results: HLA-DR4BindPred model attained superior performance on all two benchmark datasets, namely, main, and alternate over tenfold cross validation. It outperformed the existing models and improved the prediction by 13.37%, 12.27%, 11.58%, 8.55%, and 6.5% in accuracy, specificity, sensitivity, MCC, and AUC, respectively, when compared to HLADR4Pred2.0 and NetMHCIIpan4.0. It also achieved a remarkable accuracy of 98.4% and AUC of 99.5%, setting a new benchmark in HLA-DRB1*04:01 binder prediction. For the explainability of the proposed model, SHAP method was employed to analyze the feature importance and its contribution to HLA-DRB1*04:01 binder prediction.

Conclusion: We believe that HLA-DR4BindPred will significantly accelerate the identification of novel HLA-DRB1*04:01 binders, enhancing immunotherapy, vaccine design, and therapeutic strategies for diseases associated with HLA-DRB1*04:01.

C.O.I. Disclosure:

Z. Jan: None. **P.V. Jithesh:** None.

P17.100.A Unlocking genetic mysteries: AI-driven insights for variant interpretation

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Background: Identifying causative variants remains a significant challenge in genomics and is critical for improving the diagnostic yield of rare diseases. To address this, we have developed a suite of tools leveraging Artificial Intelligence (AI) to analyze genetic data and identify disease-causing variants. Through extensive benchmarking and collaborative efforts, our tools prioritize transparency and trust in AI predictions.

Material and Methods: We developed the *Suggested Diagnosis* AI system, which integrates pathogenicity, phenotypic data (as HPO terms), and familial inheritance patterns to prioritize causative variants within a Mendelian framework. This model incorporates a layer of Explainable AI (XAI) to elucidate the rationale behind its prediction results.In addition, moving beyond monogenic analysis, we designed an AI-driven layer to detect digenic and oligogenic variants. This module incorporates evidence of gene-gene interactions to improve the identification of complex genetic contributions to disease.

Results: The *Suggested Diagnosis* system (PMID:38520562, PMID:37577678) was benchmarked on six cohorts of WES and WGS data, successfully prioritizing causative variants in 85.7% of WES cases (top 20) and 100% of WGS cases (top 15). The digenic analysis module achieved a sensitivity of 73% (median rank 3) on 11 solved samples, improving to 81% on a larger manually curated dataset.

Conclusion: By enhancing precision and efficiency in genomic analysis, our solution accelerates diagnosis and improves diagnostic yield. It prioritizes monogenic, digenic, and oligogenic candidates through a hypothesis-driven approach. These modules are natively integrated into the eVai software (evai.engenome.com), an advanced solution for variant interpretation.

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P17.101.B GeM Finder: Identification of Genotype-Dependent Mismapping as a frequent source of spurious variant calls

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Background: Homologous genomic regions have long posed challenges for variant calling. The probability of homology-driven misalignment is generally computed on reference genomes. Here, we provide evidence for Genotype-Dependent Mismapping (GDM) as a possible source of spurious variant calls. We show that in GDM-prone regions, the presence of specific polymorphic haplotypes "switches" the degree of homology to the point that reads cannot be unambiguously mapped. Thus, mappability changes on an individual basis and may lead to spurious calls. We develop a tool, GeM Finder, that provides a consistent methodology to identify GDM-prone regions and assess impact.

Material and Methods: GeM Finder combines BLAT-based homology detection with dbSNP-referenced haplotype analysis to quantify mismapping probabilities. GeM Finder was applied to The Cancer Genome Atlas (TCGA) and GENIE datasets, to identify genes with elevated likelihood of genotype-dependent mismapping.

Results: Cancer-associated genes such as KTM2C, NOTCH2, STAT5A, and STAT5B showed up to 15% mismapping in highly homologous regions. For KMT2C, one of the most affected genes, GeM Finder identified 455 haplotypes in BAGE2 (KMT2C paralog) capable of inducing ≥25% mismapping, showing that specific alleles act as mapping switches. Mismapping was strongly allele-dependent, affecting variant calling reliability. In GDM-positive cases, confirmation of the variant call by long-range PCR or long-read sequencing is required.

Conclusion: This study introduces GDM as a common source of spurious variant calls, and GeM Finder as a novel framework for addressing homology-related errors. By systematically identifying GDM, GeM Finder enhances the accuracy of genomic analyses, paving the way for improved clinical applications.

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E. Bonetti: None. **Y. Zhan:** None. **G. Frigè:** None. **L. Mazzarella:** None.

P17.102.C Population-scale identification of cell state-dependent genetic regulation

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Background: Genome-wide association studies have uncovered numerous genetic variants associated with disease, but their

regulatory mechanisms remain poorly understood. While bulk RNA-sequencing has provided insights at the tissue level, single-cell data enable a more detailed view of genetic effects across different cell states. However, existing single-cell eQTL approaches either focus on manually annotated predefined cell types, or fail to scale to the population-scale cohorts. As a result, our understanding of cell state-dependence of regulatory effects remains limited.

Methods: We present G-MixMIL, an extension of our method, MixMIL [1], designed for scalable cell state-dependent eQTL mapping. G-MixMIL tests for cell state-dependent associations between a single gene's expression and genotype across individuals. By learning which cell states exhibit the strongest genetic regulation, the model identifies specific groups of cells without requiring detailed manual annotation. For calibrated statistical testing, we developed an approximate likelihood ratio test for our model.

Results: In the OneK1K sub-lymphoid compartment, G-MixMIL identified 538 eQTL effects exhibiting significant heterogeneity. Furthermore, we identified dynamic eQTL effects in B cells without relying on predefined differentiation trajectories. We will extend this approach to 4,978 UK Biobank (UKBB) individuals as part of the Cardinal Consortium, validating G-MixMIL's ability to discover sub-cell states from a population-scale dataset.

Conclusion: Our findings highlight the cell state-specific nature of genetic regulation and underscore the utility of G-MixMIL in discovering previously uncharacterized cell states. Ongoing work includes assessing overlaps with disease-associated variants and applying G-MixMIL to primary brain tissue, to understand the molecular mechanism of disease.

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P17.103.D Unravelling HR-positive, HER2-negative Breast Cancer and its Tumor Microenvironment Heterogeneity Using Spatial Transcriptomics

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Background: HR-positive, HER2-negative (HR+/HER2-) breast cancer exhibits significant molecular and cellular heterogeneity, influencing prognosis and treatment response. We applied spatial transcriptomics (ST) to dissect tumor microenvironment (TME) heterogeneity and its impact on clinical outcomes.

Methods: Spatial transcriptomics (Visium 10X) was performed on 86 HR+/HER2- of no-special-type of frozen tumor samples, and relative H&E slides were morphologically annotated. Single-cell deconvolution of ST spots was performed to increase the resolution of Visium data. Tumor and TME compartments were identified using morphological annotation, and differences in the abundance of deconvoluted cell types between two intrinsic molecular subtypes, LumA and LumB, were investigated. Weighted Gene Co-expression Network Analysis identified functional gene modules associated with relapse-free survival, validated using the METABRIC dataset.

Results: As expected, LumB tumors exhibithed worse relapse-free survival compared to LumA tumors ($p = 0.012$). Distinct spatial patterns emerged between LumA and LumB subtypes. LumA tumors were more enriched for CXCL12+ endothelial cells, naive B cells, and multiple CAF subtypes across both tumor and TME zones compared to LumB tumors, whereas LumB tumors exhibited higher infiltration of exhausted T cells (LAG3+), CXCL10+ macrophages, and lipid-associated macrophages (FABP5+) within TME regions compared to LumA tumors. Gene modules related to proliferation, metabolism, and innate immunity correlated with poorer disease outcomes. Specific immune populations derived from single-cell deconvolution mapped to different histological compartments, revealing distinct immune landscapes across tumor and TME zones.

Conclusion: These findings enhance the understanding of the TME in HR+/HER2- breast cancer, highlighting its critical role in tumor progression. Further validation is needed.

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P17.104.A ILIAD: the ERN-ITHACA registry of rare diseases with intellectual disability and anomalies of development

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Background: ERN ITHACA is the European reference network for rare malformative conditions, intellectual disabilities and neurodevelopmental disorders. ERN ITHACA has developed a "meta-registry" called ILIAD, connecting 71 HCPs, databases, and biobanks across the EU for patients with dysmorphic/multiple congenital anomalies syndromes and/or intellectual disability. Through the ERN ITHACA's expert and patient participation network, ILIAD is able to provide an infrastructure for diagnosis,

highly specialised multidisciplinary healthcare, evidence-based management, and collection of patient data.

Material and Methods: The registry is built on MOLGENIS open-source software, providing flexible data structures, user friendly data import and querying, and FAIR interfaces for programmatic data exchange. ILIAD consists of 2 components: a central, web-based registry and a network of linked satellite/client registries forming the ERN ITHACA registry federation. To date, two client installations have been successful and at least six more are ongoing. Data is modelled adhering to international interoperability standards from JRC and EJP-RD.

Results: In addition to the core registry, ILIAD includes subregistries of patients with biologically proven monogenic or genomic (chromosomal) diagnoses, under the supervision of ERN-based teams. ILIAD has adopted a data access policy, for requesting access to the data and the governance of the registry is in place to ensure compliance with applicable legal and regulatory requirements on the use of Personal Data.

Conclusion: We are well equipped to share ERN ITHACA patient data, yielding high-quality epidemiological insights and expert consensus statements, informing policy decisions that impact rare disease patients in general and care for ERN ITHACA patients in particular.

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P17.105.B The nf-core/variantbenchmarking Pipeline within the GHGA Framework

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Background: The German Human Genome-Phenome Archive (GHGA) provides a secure, scalable infrastructure for processing and benchmarking human omics data. As part of this effort, the nf-core/variantbenchmarking pipeline (https://github.com/nf-core/variantbenchmarking) was developed to benchmark small variants, indels, and structural variants in germline and somatic datasets, enabling reproducible and standardized analysis.

Material and Methods: Built with Nextflow, the pipeline ensures scalability and reproducibility across diverse environments, including local systems, HPCs, and cloud platforms. Users can benchmark using truth datasets (e.g., Genome in a Bottle, SEQC2) or custom VCF files. The pipeline supports normalization steps such as deduplication, variant splitting, filtration, and alignment correction, as well as benchmarking tools like hap.py, rtgtools, and truvari. It computes precision, recall, and F1 scores, offering detailed performance metrics.

Results: The pipeline facilitates accurate benchmarking and harmonization of variant-calling workflows, generating actionable performance insights like precision, recall, and F1 scores. By integrating with GHGA's secure infrastructure, it supports the preprocessing and benchmarking of human omics data while ensuring FAIR compliance. It is in nf-core github and It adheres to nf-core community guidelines, ensuring high-quality, reviewed code, modularity, and extensibility.

Conclusion: The nf-core/variantbenchmarking pipeline is specifically designed to accommodate varied research requirements, offering a modular approach that allows researchers to apply benchmarking methods most suitable for their analyses. GHGA's architecture is built on cloud computing infrastructures and includes an ethico-legal framework to ensure data protection compliance. GHGA enables researchers to conduct reproducible, rigorous, and secure research by standardizing bioinformatics workflows and governing reusability through harmonized metadata schemas.

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P17.106.C SVX: Population-scale merging of structural variants with tandem repeat-aware refinement

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Background: Studies of structural variants (SVs) in large population cohorts remain challenging due to high data volumes and imprecise breakpoints in low sequence complexity regions like tandem repeats (TRs), which are estimated to account for 70% of all SVs [1]. Variability among SV discovery pipelines exacerbate these difficulties, often leading to distorted merging outcomes that undermine downstream analyses.

Material and Methods: To address this issue, we developed SVX, a fast, memory-efficient SV aggregation method written in Rust. SVX utilizes proximity-based SV graphs to link SVs whose breakpoints lie within specified distance or similarity thresholds, then merging clusters while preserving accurate breakpoints. Recognizing the confounding influence of repeat-driven artifacts, SVX integrates curated TR catalogs and specialized logic for refining variant boundaries in TR regions.

Results: Benchmarking on long-read datasets demonstrates that SVX scales effectively to thousands of samples and improves resolution within TRs and other regions. SVX achieves a balance between over-merging and under-merging, preserving allelic diversity and allowing for more accurate allele frequency estimations.

Conclusion: By combining a proximity-based graph framework with TR-aware logic, SVX provides a scalable solution for multi-sample SV merging, enabling the generation of population-scale SV callsets.

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P17.107.D Evaluating regulatory variant effect prediction models with fine mapped chromatin QTLs

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Background: GWAS studies have identified numerous genetic variants associated with complex traits, yet interpreting their functional roles within regulatory DNA remains challenging. Chromatin quantitative trait loci (cQTLs) offer valuable insights into these regulatory mechanisms, but are limited in their sample size and cell type coverage. As an alternative, machine learning models such as Enformer and ChromBPNet can predict the effects of genetic variants on chromatin accessibility in a wide range of cell types. However, systematic evaluation of these predictions has been lacking.

Material and Methods: We created a validation dataset of genetically fine mapped causal cQTLs with empirically measured chromatin effects. Firstly, we uniformly processed 2,206 raw ATAC-seq and ChIP-seq samples from 30 datasets across 10 cQTL studies, covering eight distinct cell types. Using available genotype data from the same samples, we then fine mapped 10,119 likely causal cQTL variants (posterior inclusion probability > 0.5, variant located within accessible region).

Results: We evaluated the concordance between the predicted and measured chromatin QTL variant effects with two predictive models, ChromBPNet and Enformer. The average Spearman correlation was approximately 0.7 when evaluating both models on cQTLs from matched cell types. Interestingly, the correlation decreased to 0.4–0.5 for mismatched cell types, demonstrating that the models are able to capture cell-type-specific regulatory effects.

Conclusion: We believe our validation dataset can guide the development of more accurate variant effect prediction models, which can help us decode the regulatory DNA and contribute to a deeper understanding of the biological pathways between genetic variants and molecular traits.

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K. Kuningas: None. **D. Yarish:** None. **K. Alasoo:** None.

P17.108.A QUANTITATIVE OMNIGENIC MODEL DISCOVERS INTERPRETABLE GENOME-WIDE ASSOCIATIONS

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As their statistical power grows, genome-wide association studies (GWAS) have identified an increasing number of loci underlying quantitative traits of interest. These loci are scattered throughout the genome and are individually responsible only for small fractions of the total heritable trait variance. The recently proposed omnigenic model provides a conceptual framework to explain these observations by postulating that numerous distant loci contribute to each complex trait via effect propagation through intracellular regulatory networks. We formalize this conceptual framework by proposing the "quantitative omnigenic model" (QOM), a statistical model that combines prior knowledge of the regulatory network topology with genomic data. By applying our model to gene expression traits in yeast, we demonstrate that QOM achieves similar gene expression prediction performance to traditional GWAS with hundreds of times less parameters, while simultaneously extracting candidate causal and quantitative chains of effect propagation through the regulatory network for every individual gene. We estimate the fraction of heritable trait variance in cis- and in trans-, break the latter down by effect propagation order, assess the trans- variance not attributable to transcriptional regulation, and show that QOM correctly accounts for the low-dimensional structure of gene expression covariance. We furthermore demonstrate the relevance of QOM for systems biology, by employing it as a statistical test for the quality of regulatory network reconstructions, and linking it to the propagation of nontranscriptional (including environmental) effects. Such methodology could be deployed also for individual pathways or regulatory modules, at the scale currently more tractable for mammalian organisms, including humans.

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P17.109.B Sumstat Computational Data Hub, a high-performance storage and query infrastructure for Cross-Dataset Exploration of Genomics Summary Statistics

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Background: Efficient storage and rapid querying of genomics summary statistics, such as genome-wide association studies (GWAS) and quantitative trait loci (QTL) data, are critical for investigate genes and variants linked to diseases. To address these challenges, we developed the Sumstat Computational Data Hub, leveraging TileDB, a high-performance multi-dimensional array database, to store and query terabytes of harmonized genomics association studies.

Material and Methods: By integrating TileDB column-storage engine with Python and Dask, this system offers a high-performance framework for parallelized harmonization, data exploration and genomic data processing.

This is paired with a NoSQL MongoDB to manage a metadata database. Furthermore, the system can accommodate any Python-based function that can be executed and used with Dask for parallel processing.

Results: The system was benchmarked on a 1.5 terabyte TileDB dataset storing pQTL data. Our tests showed that a single pQTL study could be extracted in approximately 7 seconds while loading the same data in txt.gz format using python and pandas took 46 seconds.

Conclusion: The **Sumstat Computational Data Hub** represents a significant advancement in genomics data management by offering an easy-to-use, scalable, and high-performance solution tailored to modern genomics workflows. Its ability to harmonize and query data across multiple genomic association datasets supports integrative analyses and accelerates discoveries in large-scale and comparative genomics studies.

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P17.110.C Application of deep learning techniques to predict diabetes: insights from the Pima Indians diabetes database

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Background: The increasing availability of health datasets has enabled the application of machine learning (ML) and deep learning (DL) in genomics and precision medicine. However, challenges persist in optimizing these techniques for diagnostic and preventive healthcare. This study explores DL algorithms for analyzing complex health data, using the Pima Indians Diabetes Database as a model to extract meaningful insights.

Material and Methods: The Pima Indians Diabetes Database from Kaggle, containing patient demographics and clinical metrics, was utilized to identify key risk factors, predict outcomes, and analyze feature importance. Data preprocessing included

handling class imbalances, normalization, and outlier detection to improve model performance. Supervised ML algorithms, such as Gradient Boosting, were used for diabetes risk prediction, while DL models like convolutional neural networks (CNNs) and recurrent neural networks (RNNs) facilitated feature extraction and classification. Comparative evaluations of model accuracy, sensitivity, and specificity were conducted, and an ensemble model combining ML and DL techniques was developed to enhance predictive performance.

Results: The CNN model achieved 94.7% accuracy, while the RNN model reached 92.5%. The analysis of the feature importance identified glucose levels, BMI, and age as critical diabetes predictors. The ensemble model, integrating CNN and Gradient Boosting, improved sensitivity and minimized false positives. Visualization methods, including heatmaps and t-SNE, highlighted high-risk patient clusters, supporting targeted interventions.

Conclusion: This study demonstrates the effectiveness of ML and DL in predictive healthcare, enhancing disease understanding and aiding precision medicine. The findings highlight computational tools' transformative potential in improving patient outcomes and addressing complex health challenges.

C.O.I. Disclosure:

M. Khaleghian: None.

P17.111.D SAVViDB: A web-based visualisation tool for enhancing splice-altering variant prediction and interpretation

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Background: Splice-altering variants (SAVs), genetic variants which disrupt pre-mRNA splicing, are a major source of pathogenic variants. Most go undetected, due largely to limitations of computational prediction methods.

Methods: We developed SAVViDB, a web application for visualising and understanding all major information related to predicting and interpreting SAVs. SAVViDB's unique visual representations enable insights into complex, multi-dimensional datasets and uncover between-dataset relationships through interactive highlighting. It supports both "exploratory" workflows (querying random regions with a single click) and "targeted" workflows (in-depth investigation of candidate pathogenic SAVs). Using SAVViDB we evaluated leading SAV predictors, identified limitations and proposed solutions.

Results: SAVViDB provided insights into the limitations of previously developed SAV predictors, and other opportunities to improve SAV discovery and interpretation. We found three pervasive issues with the leading SAV prediction tool, SpliceAI. Firstly, SpliceAI's distance constraint causes a mid-exonic "blind spot". Secondly, SpliceAI erroneously "masks" predicted losses of annotated splice sites. Thirdly, for many annotated splice sites, due to reference score errors, SpliceAI is incapable of detecting losses. The ensemble method AbSplice inherits these issues, and additionally, showed 70% lower sensitivity in detecting deep intronic SAVs, compared to its predecessor SpliceAI. For all of these issues, we identified their causes, quantified their impact, and developed solutions, which we provide as a new dataset "SpliceAI Patch".

Conclusion: SAVViDB is a novel visualisation tool for enhancing SAV prediction and interpretation, suitable for researchers, clinicians and developers. SAVViDB is freely available at https://savvidb.victorchang.edu.au/.

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S. Monger: None. **T. Day:** None. **P. Young:** None. **Y. Gao:** None. **I. Vorechovsky:** None. **E. Giannoulatou:** None.

P17.112.A MitSorter: a standalone resource for methylation-based discrimination of mitochondrial DNA ONT reads

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Background: The discrimination between mitochondrial DNA (mtDNA) and nuclear mitochondrial DNA segments (NUMTs) is crucial for variant calling, as NUMTs can confound the analysis of mtDNA mutations. As Oxford Nanopore Technologies(ONT) enables direct methylation detection, offering the opportunity to classify these sequences based on the lack of CpG methylation in human mtDNA, we developed MitSorter, a bioinformatic tool that distinguishes mtDNA from NUMTs.

Material and Methods: MitSorter processes ONT reads by integrating high-accuracy basecalling with Dorado and methylation-aware models to generate modification-aware BAM (modBAM) files. The tool assesses 5mCpG methylation at the read level, partitioning the dataset into two BAM files: unmethylated mtDNA reads and highly methylated NUMT reads. The tool was validated using the GIAB human ONT dataset.

Results: Mitochondrial-aligned reads showed a bimodal distribution. Considering all reads across three tested samples (N = 99,897), the majority (69%,N = 68,947) exhibited low CpG methylation levels (<5%), consistent with true mtDNA. A secondary peak at higher methylation levels (>60%) was observed, likely corresponding to NUMTs. The unsorted modBAM exhibited a mean 5mC fraction of 0.37 ± 0.13. MitSorter generated an unmethylated modBAM with a mean 5mC fraction of 0.0022 ± 0.0003, while the methylated modBAM had a 5mC fraction of 0.63 ± 0.28. Similar results were found for normal pancreatic tissue and for 12 private samples tested.

Conclusion: MitSorter is a reliable and efficient tool for distinguishing mtDNA from NUMTs using raw ONT data. This tool has the potential to revolutionize mtDNA variant analysis and facilitate the identification of novel NUMTs, as mtDNA-to-nuclear transfer is now considered an ongoing event, particularly in cancers.

C.O.I. Disclosure:

S.N. Cox: None. **A.N. Varvara:** None. **G. Pesole:** None.

P17.113.B UPDgraph: a bioinformatic tool to detect uniparental disomies (UPDs) using trio exome or genome sequencing data

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Background: Uniparental disomies of imprinted chromosomes can present with variable features both prenatally and postnatally, guiding the decision to perform whole exome or genome sequencing (WES, WGS) analysis prior to targeted methylation studies. We report three cases of trio WES illustrating the interest of a complementary bioinformatics pipeline for UPD detection.

Material and Methods: We analyzed two prenatal cases initially evaluated with cytogenetics and trio WES, yielding negative results with cumulative data indicating a likely UPD of chromosome 14: Kagami-Ogata and Temple syndromes. A third case exhibited progressive features of Silver-Russell syndrome, associated with maternal UPD of chromosome 7. We developed a tool, named UPDgraph, designed for the visual detection of UPD. UPDgraph needs a trio VCF file and a pedigree file as inputs and generates an ideogram as an image output. It is a simple python-based tool using minimal libraries (Matplotlib) and dependencies (Bedtools).

Results: Using UPDgraph on WES data, we efficiently identified all three UPD cases, later confirmed by MS-MLPA. UPDgraph's color-coded output enables quick visualization of UPD and differentiation between iso- and heterodisomy across affected chromosomal regions. UPDgraph displays variant origins in a compact and visually intuitive way.

Conclusion: Screening for UPD during trio-exome analysis is straightforward, enhancing the genetic evaluation of abnormal fetuses and syndromic patients. By incorporating this UPD detection tool into clinical practice, the diagnostic yield of trio WES and trio WGS cases can be significantly improved.

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P17.114.C Pancreatic Islet Transcriptomics Identify MMP2 as a Biomarker Candidate in T3cD-Linked Pancreatic Cancer

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Background: Pancreatic cancer(PC) is a highly aggressive, late-diagnosed malignancy. New-onset diabetes (T3cD) is recognized as an early sign of PC, yet the underlying molecular mechanisms remain unclear. Limited transcriptomic studies focus on peripheral blood, whereas pancreatic tissue alterations remain underexplored. GSE76895, the only dataset with pancreatic islet transcriptomics from T3cD patients, provides a unique opportunity to study molecular changes but lacks distinction between malignant, benign, and pancreatitis cases, limiting cancer-specific findings. We applied bioinformatics-driven patient classification to refine the dataset and identify key transcriptomic changes in T3cD-associated PC.

Material and Methods: We analyzed GSE76895 (32 non-diabetic(ND) and 20 T3cD patients) using PCA, K-means clustering, and silhouette analysis to classify malignant pancreatic cases. DEG analysis (GEO2R,p-adj < 0.05, log2FC > 1) and pathway enrichment (Enrichr) were performed. Survival (KM-Plotter) and expression analysis (GEPIA2) validated the key genes.

Results: 18 malignant ND and 16 malignant T3cD cases revealed 1,503 DEGs. Pathway enrichment analysis confirmed key pancreatic cancer and diabetes-associated processes, distinguishing T3cD-PC from non-diabetic cases and supporting the intersection of diabetes-driven pancreatic changes, metabolic dysfunction, and malignancy. MMP2 emerged as a potential player in disease progression, showing involvement in pathways linked to cellular remodeling, metabolic alterations, and tumor microenvironment changes. MMP2 upregulation was validated in TCGA-PAAD and correlated with poor survival ($HR = 1.96$, $p = 0.011$).

Conclusion: This the first pancreatic islet-focused classification of T3cD-PC patients, linking cellular remodeling, metabolic dysfunction, and tumor progression. This study demonstrates MMP2 as a potential biomarker for early pancreatic cancer detection in high-risk diabetic patients.

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P17.115.D BaCoN (Balanced Correlation Network) improves prediction of gene buffering

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Buffering between genes, where one gene can compensate for the loss of another gene, is fundamental for robust cellular functions. Understanding gene buffering can help unmask previously unidentified gene-phenotype associations and guide the development of combinatorial or genotype-specific therapeutic strategies.

While experimentally testing all possible gene pairs using perturbation techniques such as CRISPR-Cas9 is infeasible, gene buffering can be predicted genome-wide under the assumption that a gene's buffering capacity depends on its expression level and the absence of this buffering capacity primes a severe fitness phenotype of the buffered gene.

We developed BaCoN (Balanced Correlation Network), a post-hoc unsupervised correction method that amplifies specific signals in expression-vs-fitness effect correlation-based networks and quantified 147 million potential buffering relationships across 1019 Cancer Dependency Map (DepMap) cell lines.

BaCoN outperformed state-of-the-art methods including multiple linear regression, based on our newly compiled metrics for gene buffering predictions. Adding BaCoN to batch corrected or Cholesky whitened data further boosts predictive performance. We characterized a high-confidence list of 744 buffering predictions, many of which could not be predicted by any other method. While buffering paralogs mostly reside on different chromosomes, which can be understood as a plausible architecture to facilitate genomic robustness, non-paralog buffering gene pairs were more equally distributed across the genome. BaCoN performance increases with more CRISPR screens and library genes considered, making it a valuable tool for gene buffering predictions from the constantly growing DepMap.

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P17.116.A RepoGen: a scalable Python-based framework for genomic-driven target prioritisation and drug repurposing

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Background: Drug discovery is an expensive and time-consuming process, with >90% of drug candidates failing in clinical trials. Interestingly, therapeutic compounds with genetic evidence of disease association are more likely to succeed. Genome-wide

association studies (GWAS) have transformed our understanding of the genetic architecture of complex disorders and have shown promise in identifying prioritised targets within disorder-associated regions, opening the doors for enhanced genomic-driven drug discovery and repurposing through bioinformatics tools.

Material and Methods: Our framework combines several analytical approaches, including gene-drug enrichment analyses, endophenotype Mendelian randomisation to identify causal proteins, and negative correlation testing to probe established and investigational drug candidates. Unlike standalone tools, this platform also evaluates drug properties such as ADMET (absorption, distribution, metabolism, excretion, and toxicity) to rank repurposing candidates in a pathway-specific context. The tool is written in Python, built on a Snakemake framework, and packaged in a Singularity image for ease of use.

Results: To bridge the gap between genomic insights and translational drug discovery, we developed a scalable, Python-based platform that integrates multiple bioinformatics methodologies for target prioritization and drug repurposing. It offers several advantages over existing tools, including modularity, scalability for large-scale genomic and pharmacological datasets, compatibility with machine learning frameworks, and seamless integration of additional methodologies and datasets, including information on rare and structural variants.

Conclusion: RepoGen is a versatile and user-friendly framework that streamlines the identification of genetically supported drug targets and repurposing opportunities, offering researchers a robust resource to harness genomic data for precision medicine.

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I. Andrushevich Petukhov: None. **G. Breen:** None. **J. Coleman:** None.

P17.117.B Host genetics and clinical settings in COVID-19 : predictive models by machine learning algorithms

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Background: COVID-19 has made a tremendous impact worldwide, stressing the healthcare system. Frontline workers had to manage in a short time many patients with no tools to prioritise those at high risk. The unpredictable course of the disease has shown the importance of individual genetics in determining clinical phenotypes. In this context, we aim to employ an AI-based approach that includes also specific genetic variants to help unravel the architecture of such complex diseases.

Material and Methods: We refined the best predictive model for COVID-19 mortality integrating 18 genetic and 12 clinical features, by a retrospective multicentric study. We trained three machine learning models (GBM, XGB, RF) on a dataset of 532 COVID-19 hospitalised Italian patients, recruited before vaccine availability.

Results: All the models reached great values for accuracy, AUROC, f1, f2 and PR-AUC metrics. Although GBM's PR-AUC optimization resulted in a better performance (0.473 ± 0.21), we chose to deeply analyse GBM's f1 optimization that provides fewer false negatives ($N_{f1} = 27$ versus $N_{PR\text{-}AUC} = 46$), being our main goal to answer. We delved into the feature importance to understand which features contribute to the model's decision: concerning the top 10, more than 50% was attributable to age, and the remainder was almost equally divided between genetic and clinical features. *HLA-DRA*rs3135363, *IL6*rs1800795, *ACE2*rs2285666, *CRP*rs2808635 globally accounted for 20%, demonstrating genetic data didn't confound, but implemented the model.

Conclusion: Integrating genetic and clinical data in intelligent systems is crucial for identifying high-risk cases, enhancing the management of hospitalised patients and preventing severe progression, increasingly enabling P4-medicine.

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P17.119.D Prioritization of non-coding variation using improved DNA foundation models OR why is my model so bad at rare variants?

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Background: Foundation models that predict chromatin accessibility or gene expression directly from DNA-sequence are highly performant, and attributions ascribed by these models identify relevant sequence features such as enhancers and TF binding sites. Absent fine-tunning, however, these models perform poorly on genetic variation, and even with fine-tunning fail to generalize. We focused here on a core question: Do models improve when they focus on rare variants that are more likely to disrupt fundamental features of regulatory sequence?

Material and Methods: We created a model (**Forzoi**) based on an optimized Borzoi architecture using Flash Attention and rotary embeddings that allows for a 10x-64x speed up in the evaluation of genetic variants. We used this model for zero-shot predictions of functional genetic variation across a range of allele frequencies using allele-specific open chromatin data and compared these results to models fine-tuned on the same data using a contrastive loss function.

Results: Despite having large effect sizes and more frequently contributing to allelic imbalance, rare variants were not detected zero-shot with greater performance than common variants. But while false negative rates were high, the effect sizes and directionality of predicted variants was correct with little evidence for "sign-switching" as observed in other models. Fine-tunning substantially improves performance and generalizes well to unseen genomic regions.

Conclusion: Fine-tunning on genetic data enriched for high effect, rare variants improves model performance and is generalizable. The resulting model can be (and has been) used for

predicting and interpreting medically relevant non-coding variation. Things are not hopeless.

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P17.120.A 10 years of the Open Targets Platform: bringing the genetics of monogenic and complex traits together to identify and prioritise targets for drug discovery

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Background: The Open Targets Platform (https://platform.opentargets.org/) was established 10 years ago to provide an open resource to the research and industry community for systematic gene-disease association evidence to prioritise targets for drug discovery1. It now unifies the genetics of monogenic and complex traits, with additional layers of data and analysis to refine therapeutic hypotheses.

Material and Methods: >20 resources are integrated to support target-disease associations. Genetic evidence includes; predicted causal genes underlying complex traits through our Locus2Gene pipeline harnessing GWAS and functional genomics data2, gene burden analysis utilising rare variation from whole exome/genome analysis, rare disease clinical annotations. A gene-disease prioritisation tool ranks the associations based upon an evidence scoring framework for each data source, adjustable by users, and targets can be further prioritised based on clinical precedence, tractability and safety.

Results: The Platform associations page brings evidence from rare and common disease and genetics sources into one view to help unify underlying biological mechanisms. New variant pages display evidence from complex genetic analysis providing GWAS/molecular QTL credible sets alongside clinical and pharmacogenetic associations, and direction of effect annotation. This provides the ability to build evidence for the mechanism of a genetic variant across rare and common traits potentially connected, and can provide stronger evidence for the causal role of a gene in a given disease.

Conclusion: The Open Targets Platform provides a comprehensive resource for gene-disease association evidence to help prioritise targets for the development of safe and effective treatments for common and rare diseases1.

C.O.I. Disclosure:

E. McDonagh: None.

P17.121.B Molecular Dynamics Insights into SLC35A2: Unraveling Dimerization Interfaces and Pathogenic Mutations in MOGHE and CDG

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Background: SLC35A2 is a UDP-galactose transporter that plays a critical role in cellular glycosylation, yet its structural and functional mechanisms remain poorly understood due to the lack of experimental models. Mutations in SLC35A2 are associated with congenital disorders of glycosylation (CDG) and mild malformations of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE).

Material and Methods: We employed in-silico approaches to investigate the structural dynamics of SLC35A2. An AlphaFold-generated model of SLC35A2 was subjected to molecular dynamics (MD) experiments using NAMD QwikMD for equilibration and all-atom simulation. We also performed coarse-grained MD in membrane environments with Martini/Gromacs to explore the SLC35A2 homodimerization mechanism and its heterodimerization with SLC35A3. In silico mutagenesis was performed on SLC35A2 mutations identified in a MOGHE cohort and CDG patients to assess their impact on protein structure and function.

Results: We developed a reliable in-silico model of SLC35A2 and shed light on its conformational transitions during UDP-galactose transportation. We identified homo- and heterodimerization interfaces and revealed how specific mutations disrupt transporter stability and interactions.

Conclusion: Our computational investigation clarifies how SLC35A2's dynamic architecture and dimerization interfaces foster efficient UDP-galactose transport. More importantly, it highlights how specific pathogenic mutations can compromise this mechanism, offering a molecular rationale for glycosylation-related disorders such as MOGHE and CDG. By pinpointing regions critical for stability and function, these insights provide a valuable framework for targeted experimental validation and pave the way for therapeutic strategies aimed at rectifying glycosylation defects.

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P17.122.C GA4GH phenopacket-driven characterization of genotype-phenotype correlations in Mendelian disorders

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Background: Comprehensively characterizing genotype-phenotype correlations (GPCs) in Mendelian disease would create new opportunities for improving clinical management and understanding disease biology. However, heterogeneous approaches to data sharing and analyses have hindered progress in the field.

Material and Methods: We developed Genotype Phenotype Evaluation of Statistical Association (GPSEA), a framework for comprehensive characterization of GPCs. GPSEA ingests case-level data about individuals formatted as Global Alliance for Genomics and Health (GA4GH) phenopackets and visualizes the most salient aspects of the individuals. The visualizations guide assignment of each individual into one of (typically two) genotype classes based on disease diagnoses, sex, or genomic variants. Then, correlations with four main phenotype kinds can be tested: categorical, continuous, severity scores, and survival. We developed an independent filtering strategy to boost statistical power in testing of the categorical phenotypes encoded into Human Phenotype Ontology terms. GPSEA enables analysis of continuous phenotypes, such as metabolite levels as well as of the common clinical severity scores. Finally, the genotypes can be tested for correlations with disease or clinical manifestation onsets, or mortality.

Results: We applied GPSEA to 83 cohorts with 6059 previously published individuals with case-level phenotypes and genomic variants in one of 78 genes and 120 Mendelian diseases and identified 229 significant GPCs, with 43 cohorts having at least one statistically significant GPC. We provide GPSEA as a Python package for use in interactive computing environments.

Conclusion: These results highlight the power of standardized representations of clinical data for scalable discovery of GPCs in Mendelian disease.

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P17.123.D Phenotype extraction from clinical descriptions using Large Language Models

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Background: Standardized phenotypic descriptions are crucial for accurate patient diagnosis in healthcare. Clinicians often find it challenging to manually identify these descriptions from scientific literature or patient records. Large Language Models (LLMs) show promise in automating the extraction of textual descriptions and mapping them into Human Phenotype Ontology (HPO) terms.

Material and Methods: We present an approach for phenotype extraction that leverages LLMs. The approach comprises Concept Recognition (CR), to identify candidate concepts in unformatted content and Entity Linking (EL), to map the descriptions to HPO terms. The approach combines GPT-4 for CR and PhenoBERT [PMID:35471885] for EL, addressing limitations of previous tools. To further improve EL, we introduced a method based on Knowledge Graphs (KG) embeddings. This extension allows overcoming the need of resources for fine-tuning and retraining models with every HPO update. An expert curated the ground truth of phenotypes, for evaluating our tool performances on a benchmark dataset of 76 scientific papers describing 488 patients with digenic and oligogenic variants from OLIDA [PMID:35411390].

Results: On average, four phenotypes were extracted per patient with an execution time of 1–2 minutes. Our approach achieved an exact match with ground truth in 92% of cases. Missing phenotypes account for 5%, while unnecessary phenotypes represent 3%. Work is ongoing to assess the performance of KG embeddings.

Conclusion: LLMs enable a reduction in manual curation and a faster standardization in phenotype extraction. Applied to the genomic context, they have the potential to speed up the diagnostic process.

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P17.124.A Quality management strategy for population-scale genomic data of the national genome project in Korea

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Background: A new wave of precision medicine has become an inevitable consequence of the drastic improvement in related technologies, including high-throughput sequencing, data analysis, and data sharing. Here, we introduce a population-scale whole genome sequencing (WGS) quality management system as part of the national genome project in Korea, which aims to collect high-quality genomic data for predicting and diagnosing diseases in vulnerable populations.

Material and Methods: To maintain data quality, we have embedded quality checkpoint steps and assessment pipelines in the WGS framework to ensure consistent and reliable identification of germline and somatic variants. We addressed the inspection process, which includes double SNP genotyping tests throughout the entire sequencing process. In a one-step analysis pipeline, we are harnessing 11 different tools for quality assurance.

Results: We established a comprehensive quality management framework, spanning from sample quality control to analysis-related quality control for large-scale WGS processing. This process scrutinizes various categories, including sequencing depth, sample swaps, cross-contamination, sample purity, and discrepancies between biomedical and WGS data. We also reported the statistics and representative cases of quality control for 15,000 participants in the pilot project.

Conclusion: This framework is suggested as a foundational method for executing large-scale population genomics research during phase I of the project, with plans to expand to a quarter million genomic datasets. The strategy has the potential to serve as a key element in harmonizing and integrating with other genomic research initiatives.

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P17.125.B Comparative Examples of Using Computational Structural Tools to Interpret *MUTYH* Gene Missense Variants

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Background: Mutations in the *MUTYH* gene cause DNA damage, contributing to carcinogenesis. *Insilico* analyses, leveraging various algorithms, are valuable for predicting the impact of missense variants due to time and resources efficiency.

Material and Methods: AlphaFold-generated human_MutY structure (PDB-ID:Q9UIF7) was aligned with Musmusculus_MutY-dsDNA complex (PDB-ID:7EF8). After removing the mouse structure, dsDNA and human_MutY were refined using HADDOCK, and the best-scoring model was selected. The thermodynamic effects of missense variants were assessed using PremPS, mCSM, and DynaMut2, and compared with predictions from AlphaMissense and EVE. Nineteen known pathogenic/benign variants and five variants of uncertain significance (VUS) in *MUTYH* were analyzed.

Results: Fourteen of 16 pathogenic variants were destabilizing according to at least two tools. Two pathogenic variants were destabilizing only in PremPS: Y176C as pathogenic by both AlphaMissense and EVE, R106W as pathogenic only by EVE. Three benign variants were predicted as benign by AlphaMissense and EVE, and two showing stabilizing effects. M198V, a benign variant, exhibited destabilizing effects in all tools. All VUS' were predicted as benign by AlphaMissense. EVE predicted for two VUS' as pathogenic.

Conclusion: Our analysis suggests mCSM frequently predict destabilizing effects, while PremPS and EVE demonstrate greater consistency in predicting pathogenic variants. A263G was re-evaluated based on ACMG criteria, suggesting potential pathogenicity. This study highlights the utility of computational tools in predicting the effects of *MUTYH* missense variants in a Turkish patient cohort suspected of hereditary cancer syndrome. Furthermore, it underscores the importance of caution when interpreting missense variants using *insilico* tools.

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P17.126.C Optimizing insert size ditribution in whole exome sequencing for improved variant detection

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Next-generation sequencing plays a crucial role in clinical genetics, particularly in the diagnosing of rare disorders. Whole Exome Sequencing (WES) is widely used due to its cost-effectiveness compared to targeted panels or whole-genome sequencing. Optimizing library preparation to obtain longer insert sizes has been shown to enhance mappability in challenging genomic regions, such as pseudogenes, thereby increasing variant detection.

In this study, we analyzed multiple NA12878 WES replicates by segmenting them into insert size intervals ranging from 1 to 1000 bases. Our findings confirm that longer fragments improve mappability in difficult regions and reduce false-negative variant calls.

Additionally, they contribute more effectively to mapped coverage by reducing the number of overlapping bases between mate reads, which are typically counted once when computing the mapped coverage. However, as insert size increases, a growing proportion of reads partially or fully map outside the regions of interest (ROIs), leading to reduced target coverage for the same amount of sequenced data.

While genotypability and variant calling improve with increasing fragment length, surpassing a certain threshold leads to an accumulation of near- and off-target bases, ultimately reducing the benefits for coverage depth. These findings highlight the importance of fine-tuning insert size distribution to enhance variant detection while minimizing the sequencing costs of WES experiments.

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P17.127.D VARista: a free web platform for streamlined whole-genome variant analysis across T2T, hg38, and hg19

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Background: Efficient analysis of genomic variants is crucial for advancing our understanding of genetic disorders. Existing tools, however, often pose challenges such as high complexity, delayed integration with cutting-edge resources, and significant costs. To address these issues, we developed VARista, a freely available web-based platform designed to simplify variant analysis workflow, with an emphasis on aiding research into rare monogenic diseases.

Material and Methods: VARista offers simplified analysis of VCF files from hg19, hg38, and notably, the latest T2T assembly. The platform integrates essential features for variant analysis, including comprehensive gene summaries and links to invaluable resources. It provides access to detailed tissue and cell-specific expression data, enhancing research precision. Additionally, VARista includes tools for PCR design and incorporates analyses from SpliceAI and AlphaMissense, enriching the platform with deep biological insights. Importantly, it integrates minor-allele-frequency data from the latest versions of gnomAD4 and ALFA, collectively spanning over 1,000,000 samples, thus offering a broad genomic context for variant evaluation, all aimed at enhancing the user's focus on biological insights.

Results: VARista efficiently narrows down and prioritizes variants, offering users essential biological context. This facilitates quicker, more informed decision-making in identifying disease-associated genetic variants. https://VARista.link

Conclusion: VARista emerges as a key resource for the genomic research community, combining ease of use with a robust set of features for variant analysis. By bridging the gap between complex genomic data and actionable insights, VARista empowers researchers to make better-informed decisions and accelerate breakthroughs in the understanding and treatment of genetic diseases.

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P17.128.A Latent variable analysis of kidney function genome-wide association studies enhances detection of functional variants

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Background: Chronic kidney disease affects >10% population worldwide and ranks among the top causes of death. Due to disease complexity and lack of specific biomarkers, genome-wide structural equation modeling (GenomicSEM) has emerged as a potential method for identifying molecular targets of unobservable traits that reflect a series of observed biomarkers. We applied GenomicSEM to European-ancestry GWAS summary statistics released by the CKDGen Consortium and by UK Biobank, identifying a single latent kidney function factor in which we observed enhanced detection of missense variants, compared to the underlying GWAS traits.

Material and Methods: Using approximately 6 million variants from GWAS summary statistics for eGFRcrea and eGFRcys, BUN, and UA, we employed the "commonfactorGWAS" function from GenomicSEM v0.05c to estimate the GWAS of the corresponding latent kidney function trait. Genome-wide significant signals from all the traits were identified and tested for independence using GCTA-COJO for multi-SNP-based joint and conditional analysis. Independent signals were annotated using VEP.

Results: Based on Fisher's exact test, we observed a significant increase in the proportion of missense variants among of the generated credible sets in the latent kidney function trait compared to standard traits such as eGFRcrea (P-value = 0.0004998) eGFRcys (P-value = 0.0009995).

Conclusion: Application of genome-wide SEM analysis to kidney function traits enhances the detection of functional variants for further molecular target prioritization.

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P17.129.B From genomic structure to risk prediction: Developing a CNV-based polygenic risk score for epilepsy

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Background: Polygenic risk scores (PRS) based on single nucleotide polymorphisms (SNPs) have been widely developed for predicting complex genetic disorders but often overlook structural variants, such as copy-number variants (CNVs). CNVs, particularly deletions and duplications, are known contributors to epilepsy risk, yet their role in PRS remains unexplored. We present the development of a CNV-based PRS for epilepsy using the Epi25 cohort, one of the largest epilepsy cohorts to date.

Material and Methods: Our study includes 23,049 epilepsy cases from the Epi25 consortium and 8,029 controls. Samples were genotyped using the Illumina Global Screening Array and underwent stringent CNV quality control. CNVs were categorized as deletions or duplications. The cohort was split into Preparation (~75%, N = 23,289), Training (~13%, N = 4,096), and Test (~12%, N = 3,753) datasets. Using the Training dataset, we will derive a CNV-based PRS incorporating CNV frequency, size, genomic location, and overlap with epilepsy-relevant genes. The PRS will be validated in the Test dataset.

Results: Preliminary analyses confirm high CNV call quality, with balanced CNV representation in cases and controls. CNV burden is significantly enriched in epilepsy subtypes, particularly for rare deletions in hotspot regions and duplications spanning epilepsy-associated genes. Our dataset is well-suited for developing a CNV-based PRS, with early findings suggesting its potential to enhance risk prediction beyond SNP-based approaches.

Conclusion: This study pioneers a CNV-based PRS for epilepsy, complementing SNP-based models and highlighting the unique contribution of CNVs. Our findings underscore CNV-based PRS as a promising tool for predicting epilepsy risk and understanding its genetic architecture.

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P17.130.C ANTARES (AdvaNced Technologies for genomics in rARe pEdiatric disorderS): automated AI-driven pipeline for clinical and genetic database creation

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Background: Creating structured and high-quality clinical databases is crucial for advancing medical research and improving patient care. However, manually entering information from clinical reports into such databases is both time-consuming and error-prone. Leveraging artificial intelligence (AI) and natural language

processing techniques, including the extraction of Human Phenotype Ontology (HPO) terms, offers an innovative solution for automatically generating databases from clinical reports. Furthermore, this solution allows for the automatic association of the extracted clinical data with the genetic data contained in the vcf files resulting from the genome analysis.

Material and Methods: To address these challenges, we developed an automated pipeline that utilizes AI to populate a clinical and genetic database. Starting with a clinical report written in Italian, the pipeline translates it into English, extracts the HPO terms, and converts this information into JSON files. Lastly, from the identified cases, only variants of interest are extracted from vcf files and annotated.

Results: The automated approach significantly reduced the time and cost associated with the database population, improving the accuracy of the stored information. The pipeline ensures consistency and adaptability for future structural changes. The resulting database also allows the reanalysis of inconclusive cases after discovering new genes and enables the identification of cases with phenotypes suitable for specific studies.

Conclusion: This method suggests the potential of an AI-driven database population in genetic research. It offers a scalable, efficient, and reliable alternative to manual curation. By improving data accessibility and enabling advanced analysis, this approach might support ongoing advancements in genomics.

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P17.131.D A bioinformatics approach to evaluating familial relationships through genetic similarity at selected SNP sites

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Background: Establishing family relatedness is a critical aspect of genetic studies, enabling verification of biological parenthood and the presence of consanguinity between parents. Reliable tools and methodologies are essential to ensure accurate results, particularly in clinical and research contexts.

Material and Methods: We implemented a bioinformatic procedure using bcftools and Somalier (PMID 32664994), a novel tool for relatedness assessment based on a curated set of Single Nucleotide Polymorphisms (SNPs). Individual VCFs of family members are merged into a single dataset, setting missing variants to homozygous reference calls, and then split back by sample. Somalier is subsequently applied to compute relatedness between family members, with ad hoc established thresholds on different relatedness metrics based on the number of shared homozygous and heterozygous sites. The procedure was validated using an internal dataset comprising different genomic data types, including WGS, WES, and clinical WES of familial cases. This validation focused on identifying parent-proband relationships and detecting consanguinity up to third-degree relatives between parents.

Results: On a dataset of 151 sample pairs, with the established cut-off values, we achieved high performance for parental verification with 100% specificity and 99.2% sensitivity. For consanguinity detection, specificity reached 97.9%, while sensitivity was lower at 47.1% on 64 pairs.

Conclusion: Parental verification is accurate, making the procedure suitable for clinical applications. Consanguinity checks require further optimization to improve sensitivity. Future efforts will focus on refining the methodology and expand this validation.

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P17.132.A miR-RF: a machine learning (ML) algorithm for the prioritisation of clinically relevant SNVs in miRNAs

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Background: MicroRNAs (miRNAs) are short non-coding RNAs that downregulate gene expression either through translational repression or by inducing the degradation of their target mRNAs. Accurate annotation of miRNA genes is critical for studying their molecular functions and implications in human disorders. Currently, miRBase and mirGene are the core resources for the annotation of miRNAs. However, mirBase reports 1917 miRNAs genes for Homo sapiens, while mirGene only 508. We developed miR-RF, a computational method for the annotation of miRNAs, to: 1) assess the functional activity of miRNAs in mirBase and MirGene; 2) study the effect of single nucleotide variants (SNVs) on miRNAs, in the healthy population and disease.

Material and Methods: miR-RF was developed in Python (v3.9) and R (v4.1.2). The Caret package was used to provide ML-algorithms. miRNAs sequences were retrieved from MirGene (v3) and mirBase (v22). To evaluate the impact of SNVs miRNAs were mutated in-silico.

Results: miR-RF outperformed other computational methods for the annotation of miRNAs: 93% of MiRGene entries (471) and 63% of mirBase entries (1178) were classified as "bona-fide" miRNAs. SNVs predicted to deactivate miRNAs were under-represented (p-value 6.7e-10) in the general population, indicating negative selection. Applied to somatic SNVs in cancer, miR-RF identified several potential disruptive mutations in known oncosuppressor miRNAs. All these miRNAs were downregulated, compatible with negative selection in cancer. Similar patterns of "inactivation" and downregulation were also observed for miR-1182 and miR-664b, not previously linked with tumors.

Conclusion: miR-RF accurately annotates miRNAs and facilitates the identification of clinically-relevant mutations and miRNAs.

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P17.133.B A global expression profile of bladder cancer in early stage: a meta-analysis reveals new promising therapeutic targets

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Background: Bladder cancer (BC) is the fourth most common type of cancer in men. Several types of BC are classified by the type of cancerous cell in the tissue. Ta, papillary tumor without invading the bladder wall, can be considered for targeted therapy rather than resection since the recurrence is common. In this study, a meta-analysis was performed to find the differentially expressed genes impaired in Ta BC.

Material and Methods: Studies related to Ta were found in NCBI-GEO (GSE120736, GSE128959, GSE13507, GSE146870, GSE32548, GSE32894). Ta and adjacent samples (as controls) were selected from each study, and their expression profiles were pooled after excluding the poor-quality samples. Using the ARSyNseq (ASCA Removal of Systematic Noise for sequencing data) method implemented in the NOISeq package, batch effects were removed. The Limma package was utilized to identify meta-differentially expressed genes (Meta-DEGs). Finally, using the Enrichr database, Meta-DEGs were enriched.

Results: 1947 and 2133 Meta-DEGs were down-regulated (top: NDUFS6, VDAC2, TRUB2, SLC17A5, FDXR) and 2133 up-regulated (top: XPNPEP3, AKR1D1, LILRB1, AIRE, TACSTD2), respectively. GO terms enriched by down-regulated genes indicate that immune system-related pathways are suppressed in Ta. Significantly, GO terms enriched by up-regulated genes indicate a dramatic increase in cell proliferation, differentiation, progression of cancer, and migration.

Conclusion: Our findings suggest to develop novel methods to monitor early tumors and recurrence, and to achieve personalized therapies. Clinical trials are performed to explore the effectiveness of inhibitors of immune system components, and the key Meta-DEGs detected in this study can be promising targets.

C.O.I. Disclosure:

Z. Piryaei: None. **R. Dalirfardouei:** None. **F. Piryaei:** None.

P17.134.C When AI and whole-genome sequencing collide: shaping the future of healthcare in the era of precision medicine

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Background: Personalized medicine relies on genetic testing as a cornerstone for improving diagnostics, treatment selection, and disease prevention. Integrating whole genome sequencing (WGS) with machine learning offers unprecedented opportunities to refine clinical decision-making, optimize therapeutic strategies, and enhance patient outcomes. By analyzing vast genomic datasets, it becomes possible to tailor interventions with greater precision, ultimately transforming modern healthcare.

Material and Methods: Machine learning algorithms were applied to develop classification models based on nutrigenetic analysis, enabling personalized dietary recommendations aimed at preventing prevalent multifactorial diseases. Furthermore, pharmacogenomic profiling was conducted using the UMAP and HDBSCAN algorithm, providing comprehensive insights into Croatian population-specific drug response variations. A statistical model for molecular tumor classification was also developed using over 20,000 samples, facilitating the molecular diagnosis and targeted treatment of secondary neoplasms of unknown primary origin.

Results: Preliminary validation of nutrigenetic, pharmacogenetic, and oncogenetic machine learning models indicates that integrating WGS with machine learning significantly enhances predictive accuracy, improving preventive, diagnostic, and therapeutic protocols. Ongoing clinical validation across diverse patient cohorts aims to confirm the robustness and real-world applicability of these models.

Conclusion: These findings underscore the necessity of integrating nutrigenetics for the prevention of multifactorial diseases, pharmacogenetics for individualized treatment, and oncogenetic machine learning models for precise diagnosis and targeted therapy of malignancies. As precision medicine continues to evolve, the seamless incorporation of genomic data and artificial intelligence will drive innovation, leading to more effective treatments and improved healthcare efficiency.

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P17.136.A Comparison of the probability of mtDNA deletions in regions flanked by different combinations of direct and inverted repeats

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Background: Accumulation of large-scale somatic mitochondrial deletions is associated with both healthy aging and age-related mitochondrial encephalomyopathies. Deletions are often flanked by direct nucleotide repeats and several hypotheses were raised to explain the deletion formation with the participation of the direct repeats. Here, we hypothesized that beside the direct repeats inverted repeats may play an important role structuring mtDNA.

Material and Methods: Using a collection of human mtDNA deletions and global and local mtDNA properties (distribution of direct and inverted repeats) we revise the risks of somatic mtDNA deletion.

Results: Comparison of the probability of mtDNA deletions in regions flanked by different combinations of direct and inverted repeats uncovering that, in addition to direct repeats, which are known to affect deletions, there is a strong impact of the secondary structure of single-stranded mtDNA during replication. The secondary structure while replication is shaped by inverted repeats which can form stems and shorten the effective distance between two direct repeats, thereby increasing the possibility of deletions.

Conclusion: Our results support a recent discovery that mtDNA deletions can originate through replication slippage. An understanding of the mechanisms of deletion formation allows to predict the genetic risks of mtDNA deletions for different human haplogroups - first examples being D4a and D5a haplogroups characterized by increased longevity.

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V. Shamanskiy: None. **K. Popadin:** None.

P17.138.C Evaluation of Twist CNV Backbone Spike-in Panels at different probe densities and samples coverage for large pathological CNV detection

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Background: Copy Number Variants can cause severe clinical outcomes. Array-based tests have been the gold standard for CNV detection. However, with the increasing use of Next Generation Sequencing (NGS), detecting large CNVs through Whole Exome Sequencing analysis is becoming more appealing. This study aimed to validate an optimized method for detecting large CNVs using Twist WES panels paired with newly designed CNV Backbone Spike-in Panels.

Material and Methods: Twist CNV Backbone Spike-in Panel was added to the Twist Exome 2.0 plus Comprehensive Exome Spike-in Panel and tested on 43 Coriell samples with array-confirmed CNVs. Three different probe densities were tested: 25 kb, 50 kb, and 100 kb. The 43 test samples and 12 additional reference samples were sequenced on Illumina NovaSeq 6000. To simulate different sequencing coverages, the original reads (at mean depth of 160X) were in silico sub-sampled at various read depths, i.e. 55X and 80X. CNVs were called using the enGenome eVai Secondary Workflow, which uses a read-depth approach. To benchmark our results, 52 array-confirmed large CNVs were evaluated for each probe design.

Results: An average of 345 PASS CNV variants per sample was identified, with the highest number of variants (358) called using the 25 kb design. The sensitivity was 98.08% for all probe designs, with only one variant missed, for all tested coverages. The FPR was <1% for all three designs.

Conclusion: These results demonstrate that these new panels can effectively identify large CNVs with a sensitivity comparable to array-based methods while maintaining the SNV/INDEL detection capabilities of NGS methods.

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I. Limongelli: A. Employment (full or part-time); Significant; enGenome srl. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; enGenome srl. **V. Andrioletti:** A. Employment (full or part-time); Significant; enGenome srl. **T. Han:** A. Employment (full or part-time); Significant; Twist Bioscience. **A. Davassi:** A. Employment (full or part-time); Significant; Twist Bioscience. **S. Zucca:** A. Employment (full or part-time); Significant; enGenome. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; enGenome.

P17.139.D Creation of a population-specific genetic variation repository software system for sensitive data that incorporates Indigenous data sovereignty principles

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Background: Genome sequencing technologies have transformed the diagnosis of rare genetic disorders to allow low-cost determination of genetic variation in patients and family members. Key to the identification of causal variants is the exclusion of common variants that are unlikely to be causal for extremely rare conditions. While global shared resources, such as the gnomAD database, provide valuable insights into common genetic variation, population-specific variants are inconsistently covered. To remedy this, national and population-specific data collections have been created that report the frequency of observed DNA sequence variants in the target population. To meet the commitment to maintaining data sovereignty recognized in the United Nations Declaration on the Rights of Indigenous Peoples, some projects must maintain restricted access to variation data.

Material and Methods: The software, available in a public repository, is developed for use with standard databases and uses Python, Django, and React JS for the web application.

Results: To enable two such projects in Canada and Aotearoa (New Zealand), a set of software modules has been developed that can empower projects with similar data sovereignty commitments, such as the CARE principles, to access and adapt for their use. The modules support needs that arise when engaging with sensitive data, such as auditability of access, appropriate limitations on use, and the ability to integrate existing authentication tools into the application. The web application also includes clinician-focused features including a robust variant search, links to external resources, and transcript effect predictors.

Conclusion: Access provided via a GitHub repository.

C.O.I. Disclosure:

W.W. Wasserman: None. **B. Winter:** None. **B. Halliday:** None. **D. Markie:** None. **B. Hewitson:** None. **S. Hawarden:** None. **P. Wilcox:** None. **S. Robertson:** None.

P17.140.A Varaico collects genetic variants from full-text literature (www.varaico.com)

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Background: Building on the previously published Avada (Automatic Variants Database), we created a computational pipeline called Varaico to collect genetic variants from the literature fully automatically.

Material and Methods: Varaico downloads PubMed titles and abstracts and uses a machine learning classifier to find papers potentially containing genetic variants. It then downloads the full text of relevant papers directly from publishers or PubMed Central if available. In the full text, gene mentions are detected by matching words against a list of gene names. Variant mentions in HGVS notation are detected using regular expressions. Detected gene and variant mentions are linked by checking given reference sequences in the variant description against RefSeq transcripts of the gene. Matching gene-variant pairs are mapped to genomic coordinates using alignments of RefSeq transcripts to a genome assembly.

Results: In a first run up to 2024-12-16, Varaico processed 37,991,294 PubMed titles and abstracts, of which 1,400,457 were classified as potentially containing genetic variants. It downloaded 748,738 full text papers. A median of 8 gene mentions and 4 variant descriptions per paper were discovered in the full text of 331,709 papers with at least one gene and variant mention. Gene and variant mentions were combined and mapped to 3,271,528 distinct genomic coordinates, with a mean of 1.8 citing papers per variant.

Conclusion: Varaico successfully finds genetic variants in the primary literature that may aid in diagnosing patients with genetic diseases. Varaico variants are available at the UCSC genome browser track https://hgwdev-jbirgmei.gi.ucsc.edu/cgi-bin/hgTracks?hgS_otherUserSessionName=varaico-hg19 as well as at www.varaico.com.

C.O.I. Disclosure:

J. Birgmeier: None. **M. Haeussler:** None.

P17.141.B Predicting the clinical impact of copy number variations using a machine learning approach

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Jakub Styk[1,3], Monika Kubáňová[1,6], Diana Rusňáková[1,3], Juraj Gazdarica[1,3,4], Jozef Sitarčík[1,3,4], Werner Krampl[1,3], Ján Radvánszky[1,2,5], Tomáš Szemes[1,3,2]

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Background: Copy number variations' (CNVs) presence may cause problems in the function of the organism in case of disturbing of the pathways. Several bioinformatic methods for evaluating clinical significance have been developed to differentiate CNVs between benign and pathogenic. Those tools are typically based on ACMG guidelines (classification tools). However, prediction tools based on machine/deep learning approaches are still a new field which is recently developing. Therefore the determination of CNV's clinical impact is remaining challenging.

Material and Methods: We collected information and accessions of genes, regulatory elements, dosage-sensitive regions, and other CNVs from publicly available databases. Based on them the annotations for analysing CNV were created. With the help of machine learning methods and annotations for CNV created in the previous step, we developed the prediction models, which were trained on CNV records from ClinVar respectively for loss and gains.

Results: According to the created CNV annotations, we selected the annotations that best fit and have shown the best ability to predict the pathogenicity of CNV in the model. We compared the prediction power of the model with the publicly available tools for evaluating CNV.

Conclusion: Automated bioinformatic tools for CNV annotation and prediction of clinical significance have a great potential to facilitate the evaluation process by eliminating lengthy manual annotation, providing predictions, and aiding interpretation for faster and more precise diagnostics.

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M. Gažiová: A. Employment (full or part-time); Modest; Geneton Ltd. **J. Budiš:** A. Employment (full or part-time); Significant; Geneton Ltd. **T. Sládeček:** A. Employment (full or part-time); Significant; Geneton Ltd. **M. Kucharík:** A. Employment (full or part-time); Significant; Geneton Ltd. **Z. Pös:** A. Employment (full or part-time); Modest; Geneton Ltd. **O. Pös:** A. Employment (full or part-time); Modest; Geneton Ltd. **Z. Hanzlíková:** A. Employment (full or part-time); Significant; Geneton Ltd. **J. Styk:** A. Employment (full or part-time); Modest; Geneton Ltd. **M. Kubáňová:** A. Employment (full or part-time); Modest; Geneton Ltd. **D. Rusňáková:** A. Employment (full or part-time); Modest; Geneton Ltd. **J. Gazdarica:** A. Employment (full or part-time); Significant; Geneton Ltd. **J. Sitarčík:** A. Employment (full or part-time); Significant; Geneton Ltd. **W. Krampl:** A. Employment (full or part-time); Significant; Geneton Ltd. **J. Radvánszky:** A. Employment (full or part-time); Significant; Geneton Ltd. **T. Szemes:** A. Employment (full or part-time); Significant; Geneton Ltd.

P17.143.D Signaling Molecules-Mediated Genetic Landscape of Obesity: A Systems Biology Approach

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Background: Predisposition to obesity is polygenic, resulting from genetic-environment interactions. Genome-wide association studies (GWAS) have revealed numerous obesity-related loci, but existing data focuses on isolated molecular pathways. Further research is required to elucidate causal genes, their roles, and mechanistic pathways. This study aims to investigate obesity's molecular pathophysiology using computational bioinformatic tools and algorithms to integrate causal genes and variants with multi-system data, identifying key mechanisms and causes of obesity for clinical translation.

Material and Methods: We conducted integrative analyses with advanced bioinformatics tools. SNP annotation and pathogenicity were assessed using SIFT and PolyPhen-2, with signaling pathways mapping via KEGG. Genetic, functional, and pathway data were integrated to reveal interactions and clinical correlations using network analysis algorithms STRING and Cytoscape. Our computational approach focused on genetic variants affecting a broad spectrum of molecular phenotypes, validated using Benjamini-Hochberg correction.

Results: Initial analysis identified 9,863 obesity-associated SNPs with significant in silico evidence and 444,811 SNPs linked to complex signaling pathways and 25 key organokines. Signaling pathways alterations were observed in hormonal regulation (insulin resistance via apelin, adiponectin; hypertension via resistin; appetite control via leptin) and inflammation (IL-6, TNF-alpha).

Conclusion: Our network-based, multi-system integrative approach leverages GWAS data to uncover synergistic genetic interactions. Using bioinformatics-driven algorithms, we enable broader target SNP selection and assistance for pre-study planning, optimizing research design processes and cost while supporting broader hypotheses by leveraging insights from smaller-scale GWAS studies. This scalable systems biology approach enables an exploration of overlapping pathways and supports broader polygenic diseases (obesity) pathogenesis studies.

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A. Letukiene: None. **V. Gineviciene:** None.

P17.144.A cellink: A Scalable Framework for Integrating Genetic and Single-Cell Data

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Background: Integrating genetic data with single-cell omics is crucial for deciphering cellular heterogeneity, which is a key step toward advancing personalized medicine. While AnnData, a class for handling annotated data matrices, has become the standard data analysis structure used by various tools in single-cell research, there is a need for an extended framework that efficiently handles genetic data and scales to biobank-level datasets.

Material and Methods: *cellink* facilitates analysis by prioritizing the donor as the central unit and seamlessly integrating cell-level and donor-level data through its novel DonorData structure. Genetic data is efficiently loaded via *dask*, ensuring scalability for large datasets while extending AnnData with specialized genetic annotations. Crucially, genetic data and single-cell modalities remain synchronized, preserving their pairing even through filtering operations such as selecting cell types or patient-level treatments.

Results: *cellink* is a toolkit designed for streamlined analysis, offering a comprehensive set of tools for variant preprocessing, annotation, and selection, alongside specialized downstream analyses tailored for single-cell genomics. With our package, users

can easily perform genetic analysis of single-cell expression, including eQTL mapping, colocalization with disease loci and rare variant association studies. Additionally, *cellink* supports interoperability with standard genetic analysis workflows through data exports and includes dataloaders for deep learning, enabling AI-driven insights.

Conclusion: *cellink* bridges the gap between single-cell omics and individual-level genetic data by extending the widely used AnnData framework. It enables user-friendly and efficient analysis, enhances interoperability with genetic workflows, and integrates genetics with single-cell genomics to advance personalized medicine.

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P17.146.C Assessing targeted viral detection between different metagenomic tools

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Background: Human papillomaviruses (HPVs) are among the most diverse viral families that infect humans. Because of the large number of closely related HPV genotypes with similar sequences and the abundance of non-viral DNA in human-derived biological samples, it can be challenging to correctly detect HPV genotypes using existing bioinformatical tools in high-throughput sequencing data. PCR-based diagnostic detection remains a gold standard for detecting HPV infection. Recently, however, we created a k-mer-based Tversky indexing method (HPV-KITE) as a targeted metagenomic tool for HPV detection. In this study we assess the accuracy of six different metagenomic tools to detect HPV infection from shallow sequenced metagenome data.

Material and Methods: Using RNA and DNA metagenome data we assessed the performance of HPV-KITE (k-mer-based Tversky indexing, which is under development), Kraken2, Diamond, Viromescan, VirusFinder, HPVDetector and VirusSeq. Computational analyses were performed using Puhti and Mahti supercomputers of the CSC-Finland.

Results: Parameters such as processing time, scalability, accuracy, and system requirements have been assessed. K-mer-based methods are generally faster, but alignment methods show typically higher accuracies. However, the difference in the precision of the detection depends largely on the sequencing data type and quality as well as the complexities of the targeted infections.

Conclusion: Metagenomics tools are promising methods for systematic infection network estimation if strain-level infection resolution is sustained. Benchmarking, however, is needed to assess the best metagenomic tools for the best purposes and data types.

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P17.147.D Symptom based classification of copy number variations underlying the multidimensional autism spectrum disorder phenotype using machine learning methods

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Background/Objectives: The 16p11.2 region is frequently emphasized in CNV studies for the neurodevelopmental disorders. Clinical results of CNVs in this region has not been clearly established. We aimed to create specific symptom clusters of CNVs in this region by using advanced bioinformatics and statistical methods.

Material and Methods: For this study, we scanned microarray analysis results oft he 7568 cases who applied to the outpatient clinic of Medical Genetics between 2015 and 2022. We used k-Means clustering algorithm, C4.5 decision tree classifier, Random Tree classifier, Random Forest classifier, Logistic regression and Support Vector Machines algorithms as machine learning methods. Detailed symptom screening and scales were used in our study for the diagnosis of Autism Spectrum Disorder (ASD), which is closely associated with this region. Social communication, restricted and repetitive behaviors, sensory symptoms, aggression and regression were evaluated using 25 symptoms.

Results: We determined 147 cases (1.94%) carrying a 16p11.2 CNV. Body mass index and agression levels was found to be statistically significantly higher in patients with pathogenic CNVs. The ASD symptom-based study concluded that the accuracy rate of 76.60% with 25 Autism symptoms increased to 80.85% when the classification process was performed for five symptoms. Especially agression and repetitive behaviors differed between common and pathogenic CNVs.

Conclusion: We argued that there is a widespread effect in the diagnosis of ASD along the 16p11.2 region including both common CNVs and pathogenic CNVs.

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P17.148.A mapPat: tracking pathogens evolution in space and time

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Background: The COVID-19 pandemic highlighted the importance of genomic surveillance for monitoring pathogens evolution, mitigating the spread of infectious disorders and informing decision making by public health authorities. Since the need for the summarisation and interpretation of large bodies of data, computational methods are critical for the implementation of effective genomic surveillance strategies.

Material and Methods: Developed in R (v4.4.1) under Shiny (v1.9.1), mapPat uses publicly available metadata and sequences from GISAID or NextStrain to summarise key features of variants, lineages and mutations at different times and geographic levels.

Results: mapPat is an R Shiny application for the interactive visualisation of pathogens genomic data in space and time. It collects data in tabular format by processing publicly available metadata and associated sequences. Users interact with mapPat by setting filters through a dedicated control panel; once a selection is applied, tabs are populated with informative plots and the selected data can be visualised and inspected. mapPat allows the dynamic monitoring of the evolution of variants, lineages and mutations in the genome of a pathogen at glance, through informative geographic maps and elegant data visuals. The tool was tested by retrospective analysis of SARS-CoV-2 circulation in Italy.

Conclusion: mapPat may support fighting future pandemics providing a fine grained map of pathogens evolution and circulation, and thus represents a useful method for the genomic surveillance of pathogens. mapPat is available at GitHub (https://github.com/F3rika/mapPat.git) together with a dedicated Zenodo Repository (https://doi.org/10.5281/zenodo.14163899) of precomputed datasets.

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E. Ferrandi: None. **G. Pesole:** None. **M. Chiara:** None.

P17.149.B Evaluation of SNV and CNV calling on the clinically relevant high-homology gene PMS2

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Background: Variant calling is challenging in high-homology regions when using short-read sequencing due to ambiguous alignment. This impacts the analysis of clinically relevant genes like PMS2, a well-known tumor suppressor gene involved in mismatch repair, sharing high homology with PMS2CL. Having the promise of long-read sequencing to improve the accuracy of variant calling in these regions, we compared single nucleotide variant (SNV) and copy number variation (CNV) calling performances with state-of-the-art short and long-reads applications.

Material and Methods: The enGenome-eVai-Secondary-Workflow was applied to analyze samples sequenced by the MGZ Laboratory using target enrichment with both short-read (Illumina; exome-TWIST) and long-read (PacBio; custom hereditary tumor gene panel-TWIST) platforms. 20 PMS2-positive samples were selected. The short-read workflow was optimized by masking the pseudogene region, while the long-read analysis leveraging on the PacBio "Paraphase" module to accurately resolve haplotypes and call variants is ongoing.

Results: A benchmark analysis evaluated both the sensitivity and false positive rate of the two sequencing approaches (short-read and long-read). Preliminary data on short-reads showed a Sensitivity of 100%. Regarding the long-read data, we expect to equal these metrics for both SNV and CNVs, with improved specificity.

Conclusion: Calling SNVs and CNVs in high-homology regions is possible with an optimized short-read workflow, but differentiating between *PMS2* and *PMS2CL* calls still presents a limit. PacBio long-read sequencing promises to overcome the alignment issues inherent in high-homology regions. This study highlights the potential of both sequencing technologies in calling variants within challenging genomic regions, while emphasizing the importance of workflow optimization.

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V. Andrioletti: A. Employment (full or part-time); Significant; enGenome srl. **M. Sauer:** A. Employment (full or part-time); Significant; MGZ Munchen. **T. Risch:** A. Employment (full or part-time); Significant; MGZ Munchen. **A. Benet-Pages:** A. Employment (full or part-time); Significant; MGZ Munchen, Institute of Neurogenomics. **B. Klink:** A. Employment (full or part-time); Significant; MGZ Munchen. **E. Holinski-Feder:** A. Employment (full or part-time); Significant; MGZ Munchen. **E. Rizzo:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; enGenome srl. **I. Limongelli:** A. Employment (full or part-time); Significant; enGenome srl. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; enGenome srl. **S. Zucca:** A. Employment (full or part-time); Significant; enGenome srl. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; enGenome srl.

P17.150.C UCSC Genome Browser Track Decorators

Gerardo Perez, *Galt P. Barber, Jonathan Casper, Hiram Clawson, Mark Diekhans, Clay Fischer, Jairo Navarro Gonzalez, Angie S. Hinrichs, Christopher M. Lee, Luis R. Nassar, Brian J. Raney, Matthew L. Speir, Marijke J. Van Baren, David Haussler, W. James Kent, Maximilian Haeussler*

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The UCSC Genome Browser is a widely utilized web-based tool for visualization and analysis of genomic data, encompassing over 5000 assemblies from diverse organisms. Genome annotations, referred to as "features," are typically represented as colored rectangles ("exons") connected by thin lines ("introns"). This visualization tool lacked the flexibility to highlight specific feature components within a single track, often necessitating additional tracks. To address this limitation, we introduced track decorators, a feature that allows you to convey additional information on features by overlaying colored shapes and symbols on them. Track decorators support two visualization styles: "block" and "glyph." The "block" style can overlay exon and intron regions with distinct colors and labels, such as mapping protein domain boundaries onto transcripts within a single track. The "glyph" style can display symbols, such as triangles, to indicate genomic insertion sites. These decorators can be applied to BED 12+, bigBed, PSL, and bigGenePred format tracks. Ongoing advancements include the

development of Hub Space, which will provide users with dedicated storage for uploading annotation files and attaching them as track hubs. Additionally, we continue to make progress on the Liftover on the Fly feature, which enables the automatic lifting of annotations to unannotated genomes without the need for manual track lifting.

C.O.I. Disclosure:

G. Perez: Other; Modest; Kent Informatics. **G.P. Barber:** Other; Modest; Kent Informatics. **J. Casper:** Other; Modest; Kent Informatics. **H. Clawson:** Other; Modest; Kent Informatics. **M. Diekhans:** Other; Modest; Kent Informatics. **C. Fischer:** Other; Modest; Kent Informatics. **J. Navarro Gonzalez:** Other; Modest; Kent Informatics. **A.S. Hinrichs:** Other; Modest; Kent Informatics. **C.M. Lee:** Other; Modest; Kent Informatics. **L.R. Nassar:** Other; Modest; Kent Informatics. **B.J. Raney:** Other; Modest; Kent Informatics. **M.L. Speir:** Other; Modest; Kent Informatics. **M.J. Van Baren:** None. **D. Haussler:** Other; Modest; Kent Informatics. **W. Kent:** Other; Modest; Kent Informatics. **M. Haeussler:** Other; Modest; Kent Informatics.

P17.151.D Integrating Artificial Intelligence into Cancer Genomics: Opportunities and Challenges for North Cyprus

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Background: Artificial Intelligence (AI) is transforming cancer genomics by enabling personalized oncology treatments, improving early detection, and enhancing precision medicine. AI-driven approaches facilitate mutation detection, risk prediction, and therapy optimization, offering significant potential for cancer care. However, North Cyprus faces challenges such as limited genomic infrastructure, data privacy concerns, and the need for AI capacity building. This review explores AI applications in cancer genomics within the North Cypriot context, highlighting its role in tumor profiling, treatment stratification, and cost-effective cancer care.

Material and Methods: This study examines machine learning models for tumor-specific mutation analysis, AI-driven diagnostics for early detection, and predictive models for treatment response. The establishment of local cancer genomic databases and the use of federated learning systems ensure patient privacy while enabling regional collaboration. Challenges such as resource limitations, regulatory barriers, and ethical considerations are assessed, alongside best practices from global AI applications.

Results: AI-driven models improve cancer diagnosis accuracy and treatment decision-making. AI-based genomic profiling enhances personalized therapy selection, particularly for breast and colorectal cancers. A local cancer genomic database is crucial for population-specific treatment strategies. However, insufficient genomic data, limited computational resources, and regulatory hurdles remain challenges.

Conclusion: AI-driven cancer genomics has the potential to revolutionize oncology care in North Cyprus. Addressing data accessibility, infrastructure, and policy barriers is essential for sustainable implementation. This study outlines a roadmap for AI-driven cancer genomics, emphasizing strategic investments, collaborative frameworks, and policy development to enhance cancer care outcomes.

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F. Nizam: None. **P. Savaş:** None. **H. Çobanoğulları:** None.

P17.152.A Reproducibility of Genomic Variant Detection by NGS in Rare Disease Diagnostic Setting

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Background: NGS technologies have revolutionized clinical genetics by allowing rapid and comprehensive analysis of genetic data. Currently, in Türkiye, genetic clinics use whole-genome sequencing (WES) as their first-tier diagnostic test for its cost-effectiveness. As a broader test, whole-genome sequencing (WGS) is expected to replace WES in practice as costs diminish. Considering the rapid adoption of sequencing technologies in disease diagnosis, the reproducibility of variant detection is crucial.

Materials and Methods: Per RareBoost Project goals, we aim to diagnose rare and undiagnosed patients and further rare disease research and innovation. To clarify undiagnosed rare disease patients with WES-negative results, we reanalyzed these patients' previous raw data. We used the same genomic pipeline for all re/analyses: BWA for alignment, DeepVariant for SNP/indel annotation and Manta for structural variant annotation. 10 of these patients showed no causative variants and we performed WGS analysis for further investigation. The WES analyses were performed with 100X, WGS with 30X depth. We used the number of variants called as the medium for comparison.

Results: We observed a limited overlap between WES and WGS analyses on exon regions. The limited documentation for the old sequencing data and the differences between WES enrichment kits and the selection of different reference genomes are potentially the major reasons behind these differences.

Conclusion: Our results demonstrate the importance of detailed record-keeping for sequencing analyses to establish reproducibility for NGS-based diagnosis in rare diseases. These comparisons would also help understand the gain from using WGS rather than WES.

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E. Özzeybek: None. **M. Baysan:** None. **U. Özbek:** None.

P17.153.B Approximating Linkage Disequilibrium Matrices from Summary Statistics for Fine-Mapping Applications

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Human Technopole, Milan, Italy.

Background: Accurate fine-mapping relies on linkage disequilibrium (LD) matrices, but their large size and privacy concerns limit data sharing, posing challenges for collaborative studies. We propose a method to approximate LD matrices directly from summary statistics, enabling their use in fine-mapping applications. Our approach is evaluated using UK Biobank data from 30,000 individuals.

Material and Methods: We simulated various scenarios to assess the number of individuals and phenotypes required for accurate LD approximation, ensuring robustness across different genomic regions. Fine-mapping performance was evaluated by comparing the number of true positives identified using our approximated LD matrices in SuSiE against results obtained with an insample reference LD panel.

Conclusion: Preliminary findings indicate that our method reliably approximates LD matrices allowing for precise downstream applications. We aim to develop an R package to facilitate their use for fine-mapping applications. This approach could be particularly advantageous in meta-analyses, where selecting an appropriate LD reference panel is often challenging.

C.O.I. Disclosure:

G.E. de Sanctis: None. **C. Giambartolomei:** None. **N. Pirastu:** None. **S. Sharapov:** None. **F. Ieva:** None.

P17.154.C GenePath - A novel data-driven framework for reverse phenotyping

Alexander Mordhorst[1], *Peter N. Robinson*[2], *Dominik Seelow*[2], *Denise Horn*[1]

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Background: The advent of diagnostic genomics poses new challenges in respect to variant prioritization. These challenges are mainly addressed via the use of scores relying either on the prediction of variant pathogenicity or phenotypic information. The patient phenotype on the other hand is in a clinical genomics setting usually only evaluated once before genomic data has been obtained and might be incompletely captured either in relation to its full extent or in relation to the minimum amount of information necessary to in- and exclude gene variants and therefore solve the case. The process of suggesting further measures of phenotypisation, based upon genetic data - a process called reverse phenotyping - is at the moment fully based on human expert knowledge and lacks structured and data-driven frameworks, a gap we aim to address.

Material and Methods: Python, HPO, MaxO

Results: We created a model based on the HPO and MaxO ontologies which instantiates a knowledge-graph using genomic data and a-priori captured phenotypic information as inputs. This graph then can be exploited to estimate case-complexity and suggest a diagnostic route based on the information gain attached to each possible diagnostic measure.

Conclusion: Graph-based models can be used to suggest further phenotyping measures to explore an a-priori captured and maybe incomplete patient phenotype based on genomic data.

C.O.I. Disclosure:

A. Mordhorst: None. **P.N. Robinson:** None. **D. Seelow:** None. **D. Horn:** None.

P18 Large scale genomics (GWAS) and other -omics association studies

P18.001.D Multi-omics resources and long-term conditions in the Extended Cohort for E-health, Environment and DNA (EXCEED)

Chiara Batini[1,2], *Brandon Lim*[1], *Alexander T. Williams*[1], *Gerald Sze*[1,2], *Kayesha Coley*[1], *Nick Shrine*[1], *David J. Shepherd*[1], *Edward J. Hollox*[3], *Louise V. Wain*[1,2], *Martin D. Tobin*[1,2], *Catherine John*[1,2]

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Background: The Extended Cohort for E-health, Environment and DNA (EXCEED) is a longitudinal health study aimed at understanding the environmental and biological causes of long-term health conditions. It includes ~11,000 participants aged 18 and above from the Midlands (UK), who completed baseline lifestyle questionnaires, agreed to linkage of their electronic health records, and provided biological samples for genomics, proteomics, metabolomics and glycomics.

Material and Methods: Genetic data were obtained using the UK Biobank Axiom array and through imputation to the TOPMed reference panel. Ancestry groups were defined using k-means clustering on principal components and 1000 Genome Project Phase 3 (1KGP) populations as reference. Liquid chromatography and mass spectrometry were used to measure 319 proteins, 7750 metabolites and 39 glycoproteins levels. We performed association testing between genetic variants and protein levels using regenie in 1KG-EUR-like individuals, and defined cis-pQTL as the sentinel variant within 1 Mb of the cognate gene transcription start site.

Results: After standard quality control procedures, we obtained genomic data for 8499 individuals, proteomic for 1979, metabolomic for 1524 and glycomic for 900. The largest overlap was between genomic and proteomic data, including 1551 individuals, while full overlap between all omics data was available for 541 participants. We detected cis-pQTLs for 114 proteins; 59 of these proteins were not represented in UK Biobank proteomic data.

Conclusion: Here we present a comprehensive description of the omics variation observed in EXCEED, and we will discuss its impact on multiple long-term conditions.

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C. Batini: None. **B. Lim:** None. **A.T. Williams:** None. **G. Sze:** None. **K. Coley:** None. **N. Shrine:** None. **D.J. Shepherd:** None. **E.J. Hollox:** None. **L.V. Wain:** None. **M.D. Tobin:** None. **C. John:** None.

P18.002.A Mapping the protein-genetic landscape: SomaLogic versus Scanning SWATH

Michele Filosi[1], *Eva König*[2], *Solène Cadiou*[3], *Johannes Rainer*[1], *Alessia Mapelli*[3], *Giulia Pontali*[3], *Dariush Ghasemi-Semeskandeh*[3], *Michela C. Massi*[3], *Gianmauro Cuccuru*[3], *Francisco Domingues*[1], *Cristian Pattaro*[1], *Peter P. Pramstaller*[1], *Emanuele Di Angelantonio*[3], *Markus Ralser*[4], *Christian Fuchsberger*[1]

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Background: Plasma protein levels provide insight into the physiological state of individuals. Various technologies are now available for high-throughput screening of protein profiles, and numerous studies have explored the effect of genetic variants on protein expression (pQTL). However, a systematic comparison of pQTL findings across different technologies are still limited. While somalogic uses aptamer binding techology, Scanning SWATH uses mass-spectrometry, thus they provide distinct information at the protein level, which may relate to distinct underlying biological mechanisms.

Material and Methods: We measured 103 blood protein profiles in 2388 participants to the Cooperative Health Research in South Tyrol (CHRIS) study using both Somalogic and Scanning SWATH technology. Protein profiles were normalized using indirect inverse-normal transformation, removing the effects of age, sex, the top 10 genetic principal components, and use of hormonal contraceptives. Genome-wide association studies were conducted using the Regenie pipeline. Results were clumped and fine-mapped to identify independent loci, and the association results were compared at the locus-protein level.

Results: We identified 117 and 76 loci for the Somalogic and Scanning SWATH platforms respectively, 30 are common between the two platforms. After fine-mapping we found 64 loci unique to Somalogic and 31 loci unique to the Scanning SWATH dataset.

Conclusion: Despite the distinct methodologies, a significant number of genetic associations were consistently identified across both technologies. Each platform also revealed unique genetic associations that were not detected by the other. These technology-specific associations suggest that different platforms can uncover distinct biological mechanisms and pathways, which may be linked to various disease phenotypes.

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P18.003.B 10K newborn genome screening project in Qingdao, China: clinical findings and precision health insights

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Background: The 10K Newborn Genome Screening Project in Qingdao, China, aims to explore whole genome sequencing (WGS) as a tool for precision health and newborn screening. In this study, we expand on our previous pilot involving 321 newborns by reporting on the full cohort of 10,000 unselected newborns.

Material and Methods: We performed WGS at 65X depth on 9,992 newborns to analyze 245 inheritable and metabolic diseases. WGS validation was conducted on 17 mass spectrometry-positive cases, confirming 3 and identifying 43 additional positive cases. We also analyzed genomic data from 7,140 consented newborns, identifying variants, including high-confidence protein-truncating variants (PTVs), copy number variations (CNVs), and pathogenic variants. Additionally, we performed microbiome analysis, pharmacogenomic profiling of 257 genes, and genome-wide association studies (GWAS) on 42 metabolic markers.

Results: In total, 2.7% (274/9992) of newborns were screen-positive, with 40.7% carrying at least one disease mutation. Newborns carried 0–10 pathogenic variants, with most carrying 3–6. We identified 1,281 unique rare variants across 328 genes. Microbiome analysis revealed cytomegalovirus in half of the newborns, with rates varying by delivery route and birth season. Pharmacogenomic analysis identified 593,206 variants, with 45.37% novel in East Asians. GWAS identified 4,145 significant SNPs and 1,326 novel associations, including six novel missense variants linked to metabolism.

Conclusion: This comprehensive study represents the largest newborn WGS screening effort to date, providing critical insights into genetic diseases, precision health, and pharmacogenomics. Our findings underscore the potential of WGS to advance newborn screening and inform early interventions in precision medicine.

C.O.I. Disclosure:

M. Fang: None.

P18.004.C Protein Quantitative Trait Loci meta-analysis of 7,000 protein levels measured in UK INTERVAL and Italian CHRIS studies provides new insights into the genetic architecture of the human plasma proteome (Award Candidate)

Solène Cadiou[1], *Alessia Mapelli*[1], *Giulia Pontali*[1], *Eva König*[2], *Michele Filosi*[2], *Dariush Ghasemi-Semeskandeh*[1], *Brian R. Ferolito*[3], *Michela C. Massi*[1], *Gianmauro Cuccuru*[1], *Xiyun Jiang*[4], *Johannes Rainer*[2], *Peter Pramstaller*[2], *Cristian Pattaro*[2], *Alexandre Pereira*[3], *Emanuele Di Angelantonio*[1,4], *Claudia Giambartolomei*[1,3], *Christian Fuchsberger*[2], *Adam S. Butterworth*[4]

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Background: Circulating plasma proteins are critical disease markers and drug targets, yet the genetic factors influencing their inter-individual variation are not fully understood. Here we provide an updated characterization of the genetic architecture of the human plasma proteome, leveraging the 7k aptamer-based protein platform at scale for the first time.

Methods: Plasma protein levels from 7143 proteins were measured using the SomaLogic 7k assay in two European cohorts: the UK-based INTERVAL[1] study (9251 samples) and the Italian CHRIS[2] cohort (4194 samples). Genome-wide protein quantitative trait locus (pQTL) analyses were conducted independently in each study, followed by meta-analysis. We identified regional associations, performed conditional analysis to select independent signals, and tested them for colocalization across proteins. Mendelian Randomization (MR) and colocalization with 2011 traits were conducted using an external dataset comprising meta-analyses of European ancestry samples from the Million Veteran Program, UK Biobank, and FinnGen biobanks. We additionally tested our *cis*-pQTLs for colocalization with expressionQTL in various tissues.

Results: We identified 7870 significant pQTL (1784 cis and 6086 trans), including 3171 novel associations. Newly assessed proteins were less detectable and less likely to show *cis* associations compared to proteins assessed previously. 7994 significant MR associations were found, with 729 possible new opportunities of drug retargeting. 5472 *cis*-pQTLs showed at least one significant colocalization with cis-eQTLs.

Conclusion: Our study, the first of its scale across European populations, provides new actionable insights in the genetic architecture of the human proteome and gives perspective on the benefits of expanding proteomic assays for genomic research.

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P18.005.D GWAS of 59,102 glaucoma cases reveals 230 independent loci and highlights pathways involved in glaucoma

Xinyuan Zhang[1], *Neelroop Parikshak*[1], *Rujin Wang*[1], *Olle Melander*[2], *GHS-RGC DiscovEHR Collaboration*[3], *MAYO-RGC Project Generation*[3], *Penn Medicine Biobank*[3], *Colorado Center for Personalized Medicine – RGC collaboration*[3], *UCLA-RGC ATLAS Collaboration*[3], *FinnGen*[3], *UK Biobank*[3], *Giovanni Coppola*[1], *Eric Jorgenson*[1]

[1]*Regeneron Genetics Center, Tarrytown, NY, USA,* [2]*Department of Clinical Science Malmo, Lund University, Malmo, Sweden,* [3]

Glaucoma is a leading cause of irreversible blindness worldwide. Elevated intraocular pressure (IOP) is the major modifiable risk factor. Current treatments can reduce IOP but no treatments to date restore retinal ganglion cell function. Human genetic studies have the potential to identify new therapeutic targets that could address this need. In this study, we performed the largest GWAS for glaucoma to date, which consists of 59,102 cases and 1,190,670 controls. We identified 230 independent common variant loci - nearly doubling the number of loci discovered by the largest previous study (Gharahkhani, P. *et al*. 2021). Out of these independent loci, 138 our loci are novel, and 81 overlap with loci previously associated with GWAS of IOP. For example, previous studies have shown that rare LoF variation in *ANGPTL7* reduces IOP. Our increased sample size and inclusion of FinnGen enabled identification of a common variant rs147660927 [p.Arg220Cys, MAF EUR = 0.01%, MAF FIN = 4.1%] in *ANGPTL7*, which is associated with 31% lower disease risk. To further

understand the contribution of diverse molecular pathways, we used Multi-marker Analysis of GenoMic Annotation (MAGMA) analysis to search for significantly enriched pathways. Our analysis highlighted the following pathways: circulatory system development and cardiovascular system pathways ($P = 7.3 \times 10^{-9}$), molecules associated with elastic fibers ($P = 8.2 \times 10^{-8}$) and enzyme linked receptor protein signaling pathway ($P = 6.7 \times 10^{-7}$). Our study expands the spectrum of variation linking glaucoma to IOP, supports known roles of inflammation and extracellular matrix biology, and suggests new protein signaling pathways that might play a role in glaucoma.

C.O.I. Disclosure:

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P18.006.A Rare variant association study reveals small open reading frames (smORFs) as novel regulators of cardiometabolic diseases (Award Candidate)

Hyung Chul Kim[1,2], *Ruebena Dawes*[1,2], *Yuyang Chen*[1,2], *Jing Yu*[3], *Angie Bookout*[4], *Alexander Drong*[4], *Caroline Wright*[5], *Joanna Howson*[3], *Nicola Whiffin*[1,2]

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Background: Small open reading frames (smORFs) are small translated regions of ≤150 codons. They are often poorly annotated and their mechanisms remain largely unexplored. Recent ribosome profiling (ribo-seq) experiments have identified tens of thousands of novel smORFs. Here, we generated a set of high-confidence smORFs using 11 ribo-seq datasets and investigated their association with cardiometabolic traits (e.g. HbA1c and blood lipid profile).

Material and Methods: We aggregated 10 publicly available and 1 in-house ribo-seq datasets across 17 tissues and cell lines to define a set of high-confidence smORFs, defined as those found in ≥2 datasets. We then performed a rare variant association study for cardiometabolic traits from UK Biobank within these smORF regions, using custom annotations to identify smORF-disrupting variants.

Results: We identified 13,048 high-confidence smORFs. We then filtered to rare variants (MAF < 0.001) which impact the smORFs, such as start loss, stop loss and frameshift, and selected 5,993 smORFs with aggregate rare variant count >20. Using smORF-based collapsing analysis on this smORF subset, we identified 106 smORFs that are linked to cardiometabolic traits. Of these smORFs, 69 (65.1%) were located in 5′ UTR, 25 (23.6%) were located on lncRNAs, and the rest were located in 3′ UTR or out-of-frame within the CDS regions.

Conclusion: We curated a set of high-confidence smORFs and demonstrated smORFs as novel regulatory regions associated with various cardiometabolic traits. This finding underscores the potential of these regulatory elements in risk stratification and drug target discovery.

C.O.I. Disclosure:

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P18.007.B Quantifying the impact of genetic variation on healthcare cost across 1.4 million individuals from 11 studies and 7 countries: the GenCost consortium (Award Candidate)

Sebastian Marcus May-Wilson[1], *Tomoko Nakanishi*[1], *Jiwoo Lee*[2], *Padraig Dixon*[3], *Neil Davies*[4], *Andrea Ganna*[1], *Andres Vork*[5], *Lili Milani*[6], *Erik Abner*[7], *David van Heel*[8], *Stavroula Kanoni*[8], *Camiel M. van der Laan*[9], *Dorret Boomsma*[9], *Søren Brunak*[10], *Karina Banasik*[10], *Ioannis Louloudis*[10], *Daniel McCartney*[11], *Riccardo Marioni*[11], *Anne Richmond*[11], *Zhiyu Yang*[1], *Patrick Fahr*[12], *Penelope Lind*[13], *Sarah Medland*[13], *Kristina Zguro*[1], *Hamdi Mbarek*[14], *Chadi Saad*[14], *Paulus M. Torkki*[1], *Doug Speed*[15], *Jakob German*[1], *FinnGen Consortium*[16]

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Healthcare costs offer a summary measure of overall morbidity and its treatment. Quantifying the genetic contribution to these costs can inform public health policy and reveal genetic determinants of overall morbidity. We analysed healthcare costs, including inpatient, outpatient, and prescription costs, for 1.45 million individuals across seven healthcare systems with differing cost structures. Annual per-patient costs ranged widely, from €483 in the Estonian Biobank to €458,455 in the Mass General Brigham Biobank. A GWAS meta-analysis identified 53 and 183 significant loci for inpatient and prescription costs, respectively. Most effects were modest, the top inpatient locus, rs2814985 near ILRUN, was associated with a ~1% annual cost increase per allele. One-third of loci showed sex- or age-specific effects. For example, female carriers of rs62237617 near CHEK2 had 9% higher annual costs compared to 4% in males, potentially reflecting increased breast and ovarian cancer risk. Despite differences in healthcare systems, genetic correlations across inpatient studies were strong (median $rg = 0.88$), and 95% of loci showed minimal heterogeneity. PheWAS highlighted links to risk factors (e.g., BMI) and chronic diseases (e.g., asthma, depression). Using UK Biobank exome sequencing data, we found higher inpatient costs for individuals with putative loss-of-function (pLOF) variants in clinically relevant genes, including BRCA1/2, MSH2, and APC. For BRCA2 pLOF carriers, annual costs were ~1.8 times higher than non-carriers. Polygenic scores for healthcare cost also predicted costs, with the highest decile in FinnGen incurring 25% higher median costs than mid-percentiles. Genetic variation significantly influences healthcare expenditure globally, improving our understanding of morbidity.

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P18.008.C Uncovering T1D's Hidden Variants: Insights from the Largest Whole ExomeSequencing Study in Europe

Khrystyna Shchubelka[1,2], *Walter Wolfsberger*[1], *Karolina Kuliś*[3], *Joanna Polańska*[3], *Taras K. Oleksyk*[1], *Ukraine T1D Exomes Consortium*[4]

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Background: Type 1 Diabetes (T1D) genetic studies in Europe primarily focus on Western European populations, leaving Central and Eastern Europe underrepresented. Rare, endemic variants that may significantly influence T1D risk are often overlooked due to low frequency in broader analyses. This study represents the largest whole exome sequencing (WES) and genotyping effort for T1D in Europe, providing insights into previously unexplored genetic diversity, including MODY (Maturity-Onset Diabetes of the Young). Our objective is to emphasize the importance of diversity in genetic research.

Material and Methods: We established a collaborative network in Ukraine and neighboring countries, collecting approximately 11,000 samples. Of these, 2,667 T1D patients and 4,529 controls were sequenced using Twist exome capture with whole-genome genotyping. A genome-wide association study (GWAS) was performed, complemented by gene set enrichment analysis (GSEA) to identify novel T1D genetic factors based on effect size. MODY-related variants were also screened.

Results: Our analysis confirmed the highly significant roles of HLA loci (e.g., HLA-DQA1, HLA-G) in T1D ($p < 5 \times 10^{-50}$) and identified novel genetic variants unique to Eastern European populations. MODY-related variants led to the reclassification of several misdiagnosed T1D cases, highlighting the diagnostic value of genetic studies.

Conclusion: Despite challenging conditions due to the war, this study demonstrates the feasibility of large-scale genetic research in resource-limited settings. Our findings will be available as an open-access resource via the "Genomics of T1D in Ukraine" portal and ENA, supporting global research efforts and collaboration.

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P18.009.D Advancing target discovery through systematic, scalable analysis of common human variation and traits

Yakov A. Tsepilov[1,2], *Daniel Suveges*[1,3], *Szymon Szyszkowski*[1,2], *Daniel Considine*[1,2], *Xiangyu Ge*[1,2], *Irene López Santiago*[1,3], *Tobi Alegbe*[1,3], *Vivien W. Ho*[1,3], *Kirill Tsukanov*[1,3], *Annalisa Buniello*[1,3], *Ellen M. McDonagh*[1,3], *David Ochoa*[1,3]

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Background: Open Targets (OT) is a collaborative partnership between academia and industry that uses human genetics and genomics to enhance drug target identification. We present a new post-GWAS pipeline optimized for scalable, reproducible statistical genetics and genomics analyses for drug target prioritisation, significantly expanding previous OT Genetics. This update processes all publicly available GWAS from the GWAS Catalog, FinnGen, eQTL Catalogue, and UKB-PPP, making it the most extensive human common variation analysis to date.

Material and Methods: The open-source OT Gentropy pipeline leverages PySpark for ETL and runs on Google Cloud Platform or locally. It ingests multi-omic datasets, integrates GWAS fine-mapping results, and includes modules for data harmonization, ancestry-specific fine-mapping (PICS, SuSiE), colocalization (eCAVIAR, COLOC), and drug target prioritisation (L2G score).

Results: Applied to 90K GWAS Catalog studies (15% non-European), 2.7K FinnGen R12 studies, 3K UKB-PPP studies, and eQTL datasets, our analysis generated 486K complex trait credible sets (3.5× previous OTG) and 1.8M molQTL credible sets. The enhanced L2G model achieved 0.97 AUC and 0.8 average precision, identifying >358K credible sets with L2G > 0.5, resulting in over 400K gene-disease associations. Prioritised targets show enrichment for known drug targets (phase IV OR = 2.9, p = 3.8e-13).

Conclusion: Gentropy is openly available (https://github.com/opentargets/gentropy), with results integrated into the Open Targets Platform (https://platform.opentargets.org/), expanding opportunities for genetics-driven drug discovery.

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P18.010.A scDCF: A framework for detecting disease-associated cells in single-cell RNA-seq leveraging healthy panels and GWAS findings

Caicai Zhang, *Frank Qingyun Wang, Xiao Dang, Wanling Yang*

The University of Hong Kong, Hong Kong, Hong Kong.

Background: Understanding how genetic risk factors influence disease processes at the cellular level remains a key challenge in complex disease research. Identifying cellular contexts of genetic risk is critical for unraveling disease mechanisms. Advances in single-cell RNA sequencing (scRNA-seq) and genome-wide association studies (GWAS) offer powerful tools, but integrating these data types requires robust computational methods.

Material and Methods: We developed the single-cell Disease Cell Finder (scDCF), a computational framework integrating GWAS findings with scRNA-seq data to identify disease-associated cells. scDCF leverages a healthy reference panel, Monte Carlo simulations, and statistical validation to detect significant gene expression changes linked to disease-related genetic variants. This framework was applied to systemic lupus erythematosus (SLE), Sjogren's syndrome (SJS), and chronic kidney disease (CKD).

Results: scDCF identified key disease-associated cell types and subpopulations across datasets. In SLE, age-associated B cells, CD8+GZMH+ T cells, and Th1-like CD4+ T cells were enriched for interferon-stimulated genes and cytotoxic markers. In SJS, epithelial cells of serous and mucous acini exhibited heightened antigen presentation and stress responses, implicating epithelial-immune crosstalk in autoimmune activation. In CKD, proximal tubule epithelial cells displayed metabolic dysregulation and fibrosis-related gene expression, highlighting epithelial injury's role in disease progression.

Conclusion: scDCF effectively integrates GWAS-driven gene prioritization with single-cell transcriptomics to identify disease-associated cells and pathways. This approach provides insights into cellular mechanisms of complex diseases and offers a framework to study genetic risk in disease progression.

C.O.I. Disclosure:

C. Zhang: None. **F. Wang:** None. **X. Dang:** None. **W. Yang:** None.

P18.011.B Combining machine learning and single-cell omics to reveal the mechanism of action of pleiotropic variants

Camilla Marinelli, *Daniela Fusco, Mathilde André, Lucia Troiani, Martina Noè, Fabrizio Pizzagalli, Davide Marnetto, Paolo Provero*

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Background: Genome-wide association studies complemented by the analysis of genetic correlation, intermediate molecular phenotypes, and colocalization can provide valuable insight on the shared genetic determinants of complex traits. Epigenomic and transcriptomic data at the single-cell level can deepen our understanding of the molecular processes and the specific cells involved. We apply machine learning techniques to single-cell chromatin accessibility data to gain mechanistic insight into regulatory variants pleiotropically associated with body mass index (BMI) and magnetic resonance imaging (MRI)-derived brain morphology traits.

Material and Methods: Gapped k-mer support vector machines (gkmSVMs) trained on single-nucleus ATAC-seq data for 107 brain cell types were applied to credible sets of SNPs previously identified as affecting both BMI and MRI traits through gene expression (pleiotropic variants), thus identifying the cell types most likely to mediate their effects. Motif analysis and single-cell transcriptomics allowed prioritizing candidate transcription factors (TFs) involved in the mechanism through allele-dependent binding.

Results: Nine out of 128 pleiotropic variants were predicted to impact chromatin accessibility in brain cell types. For example, rs7187776, affecting BMI and caudate volume through TUFM expression, was predicted to impact chromatin accessibility specifically in microglia. Motif analysis revealed that the alternate allele creates a strong binding site for ETS TFs, while single-cell transcriptomics suggested FLI1 and ETV6 as specific candidates.

Conclusion: Combining genetic analysis and single-cell omics through machine learning provides insights into the regulatory roles of trait-associated SNPs, highlighting potential mechanisms by which pleiotropic genetic variation influences complex traits through intermediate molecular phenotypes.

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P18.012.C Five-hundred and three loci influencing immunoglobulin levels in humans

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Background: Immunoglobulins (Igs) are the effector molecules of the adaptive humoral immune system. In order to understand further the genes and pathways involved in Ig production we conducted a large-scale genome-wide association study on serum IgA, IgG, IgM and six composite Ig traits in 114,271 individuals in the UK, Iceland and Sweden. To our knowledge this is the largest population genetic analysis of serum Ig traits conducted to date.

Material and Methods: Serum Ig measurements are routinely measured in the clinic enabling genetic analysis of electronic health records. Composite Ig traits were constructed through linear transformations of primary Ig measurements and were designed to capture specific stages of B cell development such as class switching efficiency or total Ig production. High-confidence target genes were assigned using statistical fine-mapping and colocalization analysis.

Results: We uncover 503 independent associations that reach genome-wide significance for at least one trait, 450 of which are novel. We identified 309 high-confidence causal genes, many of which had no previously established role in Ig production. These have multiple different functions and specifically indicate that genetic variation in Ig levels is determined by normal B-cell development, antigen presentation, class-switch recombination, Ig synthesis and Ig homeostasis. Furthermore, 157 signals correspond to known disease loci. Particularly, we find an enrichment of rare risk variants for congenital immunodeficiencies and B-cell malignancies.

Conclusion: Our analysis provides a high-resolution view of the genes and pathways controlling humoral immunity, and a unique resource for B-cell research and clinical genetic analysis of B-cell disorders.

C.O.I. Disclosure:

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P18.013.D Shared genetic architecture of brain shape and IBD is specifically enriched for inflammatory response genes

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Background: The gut-brain axis (GBA) is a bidirectional communication network linking the central and enteric nervous systems. Its dysregulation is implicated in inflammatory bowel disease (IBD) and psychiatric conditions. Understanding its genetic basis is crucial for uncovering disease mechanisms and potential therapies.

Material and Methods: We conducted a genome-wide association study (GWAS) on cortical brain shape in 48,564 UK Biobank participants. Using the conjunctional false discovery rate (FDR), we identified shared genetic signals with a previously published IBD GWAS on 25,042 cases and 34,915 controls. We modeled genetic effects on brain morphology, performed Gene Ontology overrepresentation analysis, and conducted PheWAS to explore biological relevance of the shared loci. Additionally, we

examined directional selection based on ancient European genomes.

Results: We identified 193 shared genomic loci (1% FDR), including immune-related genes (*IL23R, IL1R2, IL10/27, SLC22A4, CARD9*). These loci were enriched for immune processes relative to all brain loci, and explained brain shape variation in regions linked to major depressive disorder. PheWAS revealed pleiotropy with immunological, autoimmune, and psychiatric traits, including schizophrenia. Two independent top SNPs increased in allele frequency from <5% to >40% in the last 9000 years.

Conclusion: These findings support the immune system's role in the shared genetic basis of brain and gut function and suggest immune-related adaptive evolution influences brain morphology. The enrichment of shared loci for inflammatory response genes reinforces links between neuroinflammation and psychiatric conditions like depression and schizophrenia, offering a foundation for further research into GBA-related diseases.

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P18.014.A Integration of multi-omics to identify regulators gene involved in multiple sclerosis disease activity

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Background: Multiple sclerosis (MS) is a complex disorder characterized by high heterogeneity in terms of phenotypic expression, prognosis and treatment response. In the present study we aimed to identify molecular markers and signatures of disease activity through the integration of multi-layer information including clinical, molecular and environmental data.

Material and Methods: 1626 Italian and French MS patients were recruited and classified according to the NEDA status (No Evidence of Disease Activity). Whole genome genotype (Infinium OmniExpress-24 array) was performed in the entire cohort. In a subset of 261 untreated MS patients we assessed the transcriptomic (mRNA-Seq), miRNA (miRNA-Seq) and methylation (Illumina EPIC array) profiles. The gene-wise scores, derived from the association of the single-omic data with the NEDA status, were then used to develop an interactome, identifying key players (regulators); pathways associated with disease activity were evaluated applying a systems biology approach (mND).

To identify transcription factors and master regulators involved in disease activity, we applied the "Genome Enhancer" software (geneXplain) to transcriptomics and epigenomics data.

Results: a gene network of ~400 genes was identified; genes (i.e. IL2RA, VCAM1) and process (i.e. IFNγ response, TNFα signalling via NFkb) related to MS were significantly enriched. Among the transcription factors and master regulators associated with MS disease activity, the most promising molecular target is STAT5B. The validation of STAT5B as master regulator in MS disease activity is ongoing through CUT&RUN experiments.

Conclusion: STAT5B seems to be involved in the regulation of genes associated with disease activity in MS patients.

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P18.015.B A transcriptome-wide association study of brain sulcal morphology in a developmental cohort

Irene Treccani[1], *Alessandro Amaolo*[2], *Camilla Marinelli, PhD Student*[2], *Mahan Shafie*[2], *Ilaria Boscolo Galazzo*[1], *Gloria Menegaz*[1], *Davide Marnetto*[2], *Fabrizio Pizzagalli*[2], *Paolo Provero*[2]

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Background: Sulcal morphology is crucial for neurodevelopment and neurodegeneration, but its genetic determinants have been investigated only in adult cohorts. Transcriptome-wide association studies (TWAS) identify trait-associated genes by imputing the genetic component of gene expression and testing it for association with measured traits. We performed a TWAS to identify genes associated with sulcal morphology in a cohort of pre-adolescents.

Material and Methods: We analyzed 4,396 European individuals from the Adolescent Brain Cognitive Development (ABCD) study. Four sulcal shape descriptors were analyzed (depth, length, width, and surface area) from 122 regions automatically segmented and labelled across the whole brain. Left and right sulci were analyzed separately and bilaterally averaged, resulting in 732 traits. TWAS was performed by associating the genetic component of gene expression in 13 brain tissues with such traits.

Results: TWAS identified 460 genes significantly associated with 561 sulcal morphology traits. Some of them, such as NSF, KANSL1, and LARS2, are located within large loci previously implicated in sulcal morphology by a GWAS of an adult cohort, thus providing independent validation and gene-level fine mapping of such associations. Newly found associations include SFTA2, previously associated by GWAS studies with autism spectrum disorder and schizophrenia, and TRIM47, associated with several other measures of brain morphology.

Conclusion: We identified genes involved in sulcal morphology measured during development, partially overlapping those identified in an independent study of an adult population and revealing new associations, thus enhancing our understanding of cortical folding genetics, with implications for neurodevelopmental and neuropsychiatric disorders.

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P18.016.C Update on the genetics of dizygotic twinning

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Background: The propensity to give birth to non-identical twins is a complex trait that runs in families. To elucidate factors regulating female fertility and infertility, we conducted a genome-wide association meta-analysis.

Material and Methods: The GWAMA included five independent studies of mothers of spontaneous dizygotic (DZ) twins (8,265 cases, and 264,567 controls) and of independent DZ twin offspring (26,252 cases, 417,433 controls).

Results: In addition to *FSHB* and *SMAD3* previously identified, we identified four new loci: *GNRH1, FSHR, ZFPM1* and *IPO8*. Of these, the first two loci have well-established roles in female reproduction whereas *ZFPM1* and *IPO8* have not previously been implicated. Significant genetic correlations were found with multiple aspects of female reproduction and body size, as well as evidence that there has been selection against DZ twinning during evolution. A DZT risk score of the 26 top SNPs from our meta-analysis in European-origin participants did a poor job of predicting twinning rate in 47 non-European populations ($r = 0.23$ between risk score and population prevalence), indicating that separate GWAS are needed in African and Asian populations to explore the causes of their respectively very high and very low DZ twinning rates. The same PGS, at the bottom end of the scale also predicted ovarian insufficiency as a cause of female infertility, suggesting a translational application of our work.

Conclusion: DZ twinning defines the top end of a continuous scale of female fertility which is strongly genetically influenced. Those at the bottom end are at markedly higher risk of anovulatory infertility.

C.O.I. Disclosure:

N. Martin: None.

P18.017.D Discovery of core genes for autoimmune thyroid disease and polyautoimmunity via genome-wide aggregation of *trans*-effects using UK Biobank proteomics study

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Background: Autoimmune thyroid disease (AITD) is the most common autoimmune disorder, often coexisting with other autoimmune diseases—a phenomenon termed polyautoimmunity. The "omnigenic" sparse effector hypothesis postulates that the polygenic effects of common genetic variants on disease coalesce via *trans*-effects on a sparse set of core genes. This study aims to identify core genes for AITD and polyautoimmunity using genome-wide aggregated *trans*-effects (GATE) analysis inspired by this hypothesis.

Material and Methods: We used pQTL summary statistics from UKB-PPP and deCODE to compute GATE scores in a target dataset (24766/451447 cases/controls) from UK Biobank. We tested for association between AITD and GATE scores, one protein at a time, and identified core genes based on the strength of the association, the number of independent *trans*-loci contributing to each score, and retained core genes with uncorrelated scores.

Results: *PDCD1* (LogOR = 0.18, $P = 10^{-138}$) emerged as the strongest. Other putative core genes with standardised LogOR>0.01 include immune checkpoint receptors *TIGIT* ($P = 10^{-119}$), *LAG3* ($P = 10^{-13}$), *LGALS9* ($P = 10^{-34}$), *CD27* ($P = 10^{-26}$), *TNFRSF9* ($P = 10^{-28}$), *TNFRSF14* ($P = 10^{-33}$), *TNFRSF8* ($P = 10^{-45}$), *LAIR1* ($P = 10^{-44}$); genes in the interferon signalling pathway *IFNL1* ($P = 10^{-95}$), *DDX58* ($P = 10^{-49}$); encode chemokines *CXCL9* ($P = 10^{-50}$), *CXCL10* ($P = 10^{-78}$), *CXCL13* ($P = 10^{-45}$), and NK cells receptors *NCR1* ($P = 10^{-29}$), *KLRB1* ($P = 10^{-14}$).

Conclusion: These results combined with findings previously reported for type 1 diabetes and rheumatoid arthritis, establish a key role for immune checkpoints in AITD and polyautoimmunity, and the first map of polyautoimmunity using core genes. They also suggest that PD-1 agonists, now being developed for rheumatoid arthritis, may help to reduce risk of other autoimmune conditions.

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P18.018.A Single-cell genomics data predicts the success of clinical trials and facilitates drug repurposing

Sodbo Sharapov[1], *Arianna Landini*[1], *Alessandro Raveane*[1], *Matiss Ozols*[2], *Stephen Watt*[2], *Cardinal Project Working group*[3], *Marc Jan Bonder*[4], *Oliver Stegle*[2,5,6], *Nicole Soranzo*[1,2,7]

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Background: Prioritizing drug targets is a critical bottleneck in pharmaceutical development. Single-cell (sc) transcriptomics can pinpoint causal genes for complex diseases by verifying if genetic variants responsible for gene expression differences are also causal to disease. We investigated whether this knowledge applied to drug target genes increases the chances of success in clinical trials and provide evidence for drug repurposing.

Material and Methods: We integrated historical clinical trial data, GWASs of 36 diseases and traits, and cis sc-eQTLs from PBMCs of ~ 5,000 UK Biobank donors across 28 cell types. First, we performed genome-wide colocalization analysis between 47,037 scRNA eQTLs and diseases. Each target-indication (T-I) pair was classified based on the presence or absence of colocalization of their eQTL with corresponding indication-related disease and compared with their success in reaching different phases of drug validation.

Results: Pairs of T-I with evidence of colocalization of target's eQTLs and disease were significantly more likely to progress through clinical trial phases compared to non-colocalizing targets: OR = 7.51 [5.21–10.50] to pass Preclinical Phase, OR = 2.75 [1.65–4.30] to pass Phase I, OR = 2.84 [1.22–5.64] to pass Phase II, and OR = 2.42 [0.66–6.26] to pass Phase III. Subgroup analyses revealed strong support from sc-eQTL for cardiovascular, gastrointestinal, and metabolic diseases. We identified multiple marketed drugs whose targets have eQTLs, colocalized with novel indications, supporting drug repurposing.

Conclusion: These findings underscore the importance of genetic colocalization in early-stage drug design. By prioritizing eQTL-supported targets, pharmaceutical pipelines can enhance clinical success rates and uncover drug repurposing opportunities.

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P18.019.B HLA PheWAS studies in the China Kadoorie Biobank and UK Biobank

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Background: The Major Histocompatibility Complex (MHC) region harbours the highest number of significant associations in genome wide association studies. However, the full phenotypic impact of the MHC locus across a wide range of diseases remain unknown.

Material and Methods: We imputed Human Leukocyte antigen (HLA) alleles from genotype data within the China Kadoorie Biobank (CKB, N>100,000) and the UK Biobank (UKB, N>500,000) using a multi-ancestry HLA reference panel. We quantified HLA diversity in CKB using a customized R package [https://github.com/yang-luo-lab/HLADiversity]. Furthermore, we performed mixed logistic regression analysis correcting for age, sex and genetic relatedness across 200 phenotypes in both biobanks.

Results: We observed 316 unique HLA alleles at 2-field resolution in the CKB dataset. HLA allele frequencies among the ten CKB regions showed distinct north-south division. From the CKB and UKB PheWAS analysis, we observed previously reported significant HLA-disease associations across various phenotypes with consistent effects across two ancestral groups. However, there exist ancestry-specific associations. For example, DRB1*04:01 is the strongest risk allele associated with rheumatoid arthritis in UKB ($\beta = 0.410$), while DRB1*04:05 confers the strongest risk in CKB ($\beta = 0.578$). Additionally, meta-analysis identified potentially novel HLA associations, including HLA-DRB1*03:01 with thyrotoxicosis hyperthyroidism ($P = 1.21 \times 10^{-140}$, OR = 1.747) and HLA-A*02:01 with shingles ($P = 1.10 \times 10^{-26}$, OR = 0.866).

Conclusion: We present findings on the influence of HLA variants on multiple phenotypes across global biobanks. Our results underscore the significant role of HLA alleles in shaping a diverse range of disease phenotypes worldwide.

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P18.020.C Missense variants in *FRS3* affect body mass index in populations of diverse ancestries

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Background: Obesity is a global epidemic associated with adverse effects on health and quality of life. Improved understanding of its underlying pathophysiology is essential for developing counteractive measures. For this purpose, our aim was to search for sequence variants with large effects on body mass index (BMI).

Material and Methods: We performed a multi-ancestry meta-analysis of 13 genome-wide association studies on BMI that included data derived from 1,534,555 individuals of European ancestry, 339,657 of Asian ancestry, and 130,968 of African ancestry. We focused on sequence variants with an absolute effect greater than 1 kg/m^2.

Results: An intergenic 262,760 base pair deletion at the *MC4R* locus associated with 4.11 kg/m^2 higher BMI per allele ($P = 4.5 \times 10^{-9}$; 95% confidence interval [CI; 2.76, 5.46 kg/m^2]), likely through downregulation of *MC4R*. A rare *FRS3* missense variant, p.Glu115Lys, was only found in Finland and associated with 1.09 kg/m^2 lower BMI per allele ($P = 6.6 \times 10^{-12}$; 95% CI [−1.40, −0.78 kg/m^2]). Three other low-frequency missense variants in *FRS3* associated with BMI with smaller effects ($P < 2.6 \times 10^{-4}$) and were enriched in different ancestries. In a gene-based burden test of *FRS3* predicted loss-of-function variants, loss of FRS3 function associated with a 2.24 kg/m^2 increase in BMI ($P = 0.0062$; 95% CI [0.62, 3.85 kg/m^2]).

Conclusion: *FRS3* is a novel BMI-associated gene that encodes an adaptor protein known to act downstream of BDNF and TrkB, which, upon activation, decrease appetite and food intake through previously unknown signaling pathways. Our work provides a convincing downstream component of the BDNF-TrkB pathway, a potential target for obesity treatment.

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P18.021.D Genetic and phenotypic underpinnings of within-genetic sex variability in human plasma proteome across 51,292 individuals and five studies

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Background: Protein expression (PEX) signatures underlying sex-specific biology remain understudied. By exploring the overlap and divergence in PEX within and between sexes, we aim to uncover mechanisms underlying sex-linked phenotypes, and their health implications.

Material and Methods: We included 51,292 individuals with 29,19 plasma proteins measured across five studies (UK Biobank, FinnGen, MESA, Biobank Japan, Japan COVID-19 Task Force). To understand proteome-wide sex-linked variability, we derived an XGBoost-based Proteomic Sex Index (PSI) comprising 31 proteins, using 9,128 individuals from UKB. High PSI indicates the proteome is shifted from the genetic sex-typical values.

Results: We observed sex-biased PEX in 80% ($p < 2e-05$) of proteins in UKB. Sex bias estimates correlated well across studies (Pearson's r: 0.72–0.80). Interestingly, 11% of proteins were differently associated with sex before versus after menopause in women.

PSI was associated with sex-differential phenotypes in meta-analyses: e.g., males with high PSI had lower testosterone (nmol/L, beta = −2.66, 95% confidence intervals:−0.32,−0.21) and were shorter (cm, beta = −0.43; 95% confidence intervals:−0.52,−0.34).

We identified five genome-wide significant loci in within-sex PSI GWASs in UKB. Two were shared (MMP3, CDH15), and the other loci with known pQTLs were XG in males and PZP in females. We calculated PSI polygenic score (PGS) within FinnGen and examined its correlations with 678 diseases. Higher PSI PGS in females is associated with a lower risk of female-biased diseases.

Conclusion: These findings reveal complex patterns of sex-biased proteomic variability, providing insights into sex-specific health risks.

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P18.022.A The host genetic architecture of Epstein-Barr virus (EBV) abundance in blood

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Background: Epstein-Barr virus (EBV) is an endemic DNA virus, with about 95% of the adult population being infected. Primary infections are usually asymptomatic in childhood and present as infectious mononucleosis in later life. EBV persists latently within lymphocytes and is associated with a variety of disorders, such as autoimmune and tumour diseases (e.g. Multiple Sclerosis (MS)). However, little is known about the host's interaction with EBV. Here, we used genome sequence (GS) data of the UKBiobank to quantify EBV abundance and analyse the host genetics of EBV-control.

Material and Methods: EBV-reads were extracted from UKBiobank GS data and the presence of EBV-reads ("EBV-read-positivity") was characterized using clinical and serology information. Genome-wide common and rare-variant association studies were performed in 360,764 European individuals using Regenie with covariates (e.g. blood count traits, age, sex, sequencing yield). The distribution of polygenic risk scores (PRS) for EBV-read positivity was compared across different disease cohorts reflecting EBV-associated traits.

Results: 78,771 of 486,315 individuals carried >=1 EBV-read. EBV-reads were practically absent in seronegative individuals (specificity: 99.5%) and strongly overrepresented in individuals with reported HIV. GWAS identified robust associations across the MHC-region and 24 non-MHC loci, including loci harboring genes involved in antigen processing. RVAS identified TNFRSF13B, underlying common variable immune deficiency, as a significant gene outside of MHC. EBV-read-positivity-PRS was significantly associated with MS and driven by the MHC region.

Conclusion: We here provide novel insights into the genetic architecture of immune control in latent EBV infection and its relevance to EBV-associated disease.

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P18.023.B Assortative mating exacerbates the impact of rare pathogenic CNVs

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Background: CNVs are linked to a spectrum of outcomes, ranging from subtle to severe phenotypic changes. We explored the distribution and impact of an individual's polygenic scores (PGSs) in different CNV backgrounds.

Material and Methods: Leveraging CNVs and PGSs for 331,522 unrelated white British UK Biobank participants, we modeled 104 established CNV-trait associations involving 38 phenotypes. Linear regressions assessed the individual and joint contribution of CNVs and PGS to phenotypic variability. We tested whether healthy cohort bias results in CNV carriers exhibiting lower PGS to counteract the CNV effect.

Results: We demonstrate an additive PGS-CNV contribution for 74 (71%) CNV-trait pairs but do not identify any significant CNVxPGS interactions, e.g. a 22.7 mmol/mol increase in glycated hemoglobin between the bottom and top PGS quintiles of 16p11.2-BP4-5 deletion carriers ($p = 1.7e-4$) paralleling higher prevalence of type 2 diabetes (13% vs 46%; $p = 0.049$). Four CNV-trait pairs show a significantly higher PGS burden in CNV carriers *vs* copy-neutral individuals, with an additional 30 nominally significant pairs, indicating that CNV carriers systematically exhibit higher PGS load (34/34; $p = 1.1e-10$). Assortative mating (AM) is a likely explanation, evidenced by the strong association between the observed CNV-PGS correlations and their predicted values by the AM mechanism ($r = 0.55$, $p = 3.9e-4$), consolidated by 51% average CNV inheritance rate of 28 testable CNVs.

Conclusion: AM leads carriers of inherited rare CNVs to accumulate higher PGS burden, exacerbating CNV's effect. This interplay contributes to variable expressivity, enabling carrier stratification and identification of those at risk for severe clinical outcomes.

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P18.024.C Genetic architecture and risk prediction of gestational diabetes mellitus in over 116,144 Chinese pregnancies

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Background: Gestational diabetes mellitus (GDM), a heritable metabolic disorder and the most common pregnancy-related condition, remains understudied regarding its genetic architecture and its potential for early prediction using genetic data.

Material and Methods: In this study, we conducted genome-wide association studies(GWAS) on 116,144 Chinese pregnancies, leveraging their non-invasive prenatal test (NIPT) sequencing data and detailed prenatal records. Based on GWAS findings, we developed polygenic risk scores(PRS) and applied an XGBoost model to predict GDM, which utilized PRS, non-genetic factors, or a combination of both.

Results: We identified 14 novel loci for GDM and 115 for five glycemic traits, with allele frequencies of 0.01–0.5 and absolute effect sizes of 0.03–0.79. Approximately 50% of these loci were specific to GDM and gestational glycemic levels, distinct from type 2 diabetes and general glycemic levels in East Asians. A machine learning model integrating PRS and prenatal records predicted GDM before 20 weeks of gestation, achieving an AUC of 0.729 with accuracy of 0.835. Compared to the phenotypic model with AUC of 0.712 and accuracy of 0.835, the combined model showed significance improvement in predictive performance, as demonstrated by DeLong's test($P < 0.05$). Shapley values highlighted PRS as key contributors.

Conclusion: This study represents the largest GWAS to date on GDM and five glycemic traits in an East Asian cohort. Our findings provide the first comprehensive insights into the genetic architecture of GDM in East Asians and introduces a model that integrates PRS with early electronic health records to enhance the prediction and classification of GDM risk.

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P18.025.D Rare variant imputation followed by exome-wide association analysis uncovers novel protein quantitative trait loci

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Background: Plasma protein levels provide insight into the physiological state of individuals, yet the genetic factors influencing protein expression are incompletely characterized. Previous efforts have concentrated on analyzing common variants[1] or investigated a limited number of proteins[2]. Rare exonic variants are enriched for impact on molecular function, yet rare variant studies are often under-powered.

Methods: Plasma protein levels of 7,281 proteins were measured in 4,194 individuals of the Cooperative Health Research in South Tyrol (CHRIS) study[3] using the SomaLogic 7K platform. Since whole-exome sequenced was only available for a subset ($n = 2,396$) of individuals, a custom reference panel was used to impute exonic variants into the whole cohort, achieving a mean accuracy of 0.91 in a validation sample. Exome-wide association analysis was performed using REGENIE. GCTA was used to identify independent signals with minor allele frequency <1%. Skat and skato tests were used to perform gene-based tests.

Results: We identified 937 and 319 independent protein quantitative trait loci (pQTLs) and gene-based associations, respectively. High and moderate impact variants were enriched in rare cis-pQTLs compared to common cis-pQTLs. Only 19% of pQTLs were novel compared to 46% of gene-level associations. For 23% of the gene-level associations, no single pQTL in the gene achieved significance. For 23 pQTLs, the variant was reported as (likely) pathogenic in ClinVar.

Conclusions: We confirm previously reported associations but also provide novel insights into rare associations with the blood proteome, highlighting the value of imputation enhanced rare variant association studies and gene-based tests.

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P18.026.A Dissecting the genomic and epigenomic landscape of children's adipose tissue

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Background: Understanding age- and tissue-specific molecular mechanisms is important for elucidating metabolic disease aetiopathology. While some studies have explored DNA methylation quantitative trait loci (mQTLs) and gene expression QTLs (eQTLs) in adult adipose tissue, their regulation in child adipose tissue has never been studied.

Material and Methods: We mapped *cis*-mQTL ($N = 288$), eQTL ($N = 349$) and transcript QTL (tQTL; $N = 349$) in primary child adipose tissue and compared the results to adult datasets to identify age-specific genetic regulation. Over-representation analysis was performed to test for enrichment of the childhood-specific eQTL-targeted genes (eGenes) in biological pathways. The molecular QTL maps were integrated with genome-wide association study (GWAS) summary statistics for adulthood and childhood obesity traits and adult metabolic traits through colocalization.

Results: Child adipose tissue showed unique characteristics compared to adult datasets, with 22.2% of the eGenes not identified in adult adipose tissue. For mQTLs, up to 45% of mCpGs were age-specific. Childhood-specific eGenes were enriched in aging, anatomical structure development, and morphogenesis pathways ($qval < 0.05$). Colocalization analysis revealed 1,449 GWAS signals colocalizing with mQTLs, 478 with eQTLs, and 480 with tQTLs, with 158 loci colocalizing across all three traits. Of 408 colocalizing eGenes, 216 (52.9%) were also reported in the Open Targets Genetics database.

Conclusion: These findings show that studying the genetic regulation of molecular traits in children can identify a large

number of signals currently missed by adult adipose tissue datasets. This contributes to a better understanding of early processes related to obesity and metabolic disease in children and adults.

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P18.027.B A systematic approach to map downstream effects of proteins on the paired plasma and urine metabolome

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Background: Proteins mediate and catalyze many metabolic functions, including enzymatic reactions and transport processes in the kidney that operate at the interface of plasma and urine. Using readouts of the proteome and metabolome, we employed hypothesis-generating genetic causal inference methods to study their molecular relationships across biofluids.

Methods: We conducted proteome-wide association studies (PWAS, 1,303 cis-pQTL protein models) for the levels of 1,296 plasma and 1,399 urine metabolites, leveraging summary statistics from genome-wide association studies of metabolites (N = 5,023). Downstream analyses included statistical colocalization to address the risk of genetic confounding due to linkage disequilibrium and a phenome-wide PWAS to explore the (patho-)physiological effects of proteins significantly associated with metabolites across 4,360 phenotypes in the UK Biobank.

Results: We identified 282 putative causal effects of proteins evenly distributed on plasma and urine metabolite levels. Colocalization analyses supported 157 of the 282 findings (posterior probability of a shared causal variant >80%). For 36 of the 61 implicated unique proteins, we identified 326 putative causal effects on health outcomes ($p < 1.8e\text{-}7$). Levels of the kidney membrane enzyme DPEP1 and the urine dipeptide metabolite prolylglycine displayed the strongest association ($p = 3.6e\text{-}293$), consistent with its role as a dipeptidase. DPEP1 was associated with 13 metabolites and 21 health outcomes including higher hemoglobin concentration and reduced hypertension incidence. Using the Open Targets database, 17 metabolites and 19 proteins were among potential drug targets in clinical trials.

Conclusion: This in vivo causal inference screening of the proteome and metabolome highlights dozens of enzymes, their associated metabolites and clinical correlates.

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P18.028.D Deep learning derived adipocyte size reveals adipocyte hypertrophy is under genetic control

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Background: Fat distribution and macro structure of adipose tissue are important factors in predicting obesity-associated diseases. To investigate the relationship between adipocyte hypertrophy, (using mean adipocyte size) and obesity-related traits, and the genetics of it, we performed the largest study of automatic adipocyte phenotyping using both histological and genetic data.

Material and Methods: Utilising visual deep learning based tools for doing semantic segmentation of subcutaneous and visceral adipose tissue histology samples (N = 2,667) in 5 independent cohorts. The pipeline was able to do rapid inference across 9,000 whole slide images, and segment over 27 million adipocytes.

Results: Estimates of mean adipocyte size were validated against Glastonbury et al. 2020 showing a high level of concordance. We show that adipocyte hypertrophy confers an adverse metabolic profile with increased levels of leptin, fasting plasma glucose, glycated hemoglobin and triglycerides. It confers decreased levels of adiponectin and HDL cholesterol. We performed the largest GWAS (NSubcutaneous = 2066, NVisceral = 1878) and subsequent meta-analysis of mean adipocyte size, we find one genome-wide significant locus (rs73184721) associated with increased 95%-quantile of adipocyte size in visceral adipose tissue. Analysing females only we find two genome-wide significant loci, one variant (rs140503338) is associated with increased mean adipocyte size in subcutaneous adipose tissue, the other variant (rs11656704) is associated with decreased 95%-quantile of adipocyte size in visceral adipose tissue.

Conclusion: We show that adipocyte hypertrophy is associated with an adverse metabolic profile. The presence of extremely large adipocytes is under genetic control.

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P18.029.C Identification and analysis of pQTL-dense regions and their connection to protein functionality

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Background: Protein quantitative trait loci (pQTL) studies reveal numerous genetic variants regulating protein expression. These variants are unevenly distributed, forming hotspots in specific genomic regions, suggesting shared regulatory mechanisms for target proteins. Understanding the distribution of genetic variants is essential for deciphering genetic functions and regulatory pathways. This study aims to map genetic-variant hotspots and investigate signal clustering and functionality within these regions.

Material and Methods: We used the results of a genome-wide pQTL study of 7289 proteins, meta-analyzing data from the INTERVAL (9657 samples) and Cooperative Health Research in South Tyrol (CHRIS) study (4225 samples) cohorts. A custom pipeline was developed to characterize signal densities across chromosomes spatially. We performed a colocalization analysis on

the identified hotspots. The results for each region are represented in a network where protein pairs are connected if any of their significant genome-wide signals colocalize. Community detection network algorithms partitioned these hotspots into distinct protein modules, potentially associated with shared regulatory mechanisms. Enrichment analysis was used to characterise the community's functionality and differences.

Results: Our study identified 22 highly pleiotropic genomic hotspots accounting for 68% of trans signals alongside 16% of our cis signals. Many corresponded to known loci, such as ABO, while others were novel. Functional studies of less-characterized, highly dense loci provided insights into their biological roles.

Conclusion: We provide a framework for identifying genomic regions that influence the expression of multiple proteins, offering new insights into the different potential roles of hotspots in regulating protein expression.

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P18.030.B Genome-wide association study of *Staphylococcus aureus* nasal carriage identifies genetic loci linked to cardiometabolic traits

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Background: *Staphylococcus aureus* is a leading cause of bacterial infection-related deaths globally. Nasal carriage with *S. aureus*, found in 30–40% of the population, is typically asymptomatic but increases infection risk. The transition from harmless colonisation to invasive infection is poorly understood and likely influenced by host factors. This study aimed to identify new genetic variants associated with nasal carriage and infection risk.

Material and Methods: Genotype data from 135,000 individuals in the Danish Blood Donor Study (DBDS) were available. A subset was classified as *S. aureus* carriers or non-carriers based on a single swab ($n = 8{,}324$), while swabs on two occasions further differentiated carriers into persistent and intermittent carriers ($n = 3{,}024$).

Results: Combined-sex GWAS on nasal carriage using REGENIE identified the lead SNP rs78440534 (beta = 0.19, $P = 0.94 \times 10^{-8}$) within *IRF8* and the sex-specific GWAS revealed targets in *CCDC85A* (rs1522687, beta = 0.24, $P = 8.92 \times 10^{-8}$) in men and *HLA-DPA1* (rs3128952, beta = 0.31, $P = 4.67 \times 10^{-7}$) in women. A *KRAS* variant (rs11047908, beta = −0.64, $P = 8.12 \times 10^{-8}$) was associated with persistent carriage and a *DSCAM* variant was associated with intermittent carriage (rs1888497, beta = 0.64, $P = 3.25 \times 10^{-8}$). These variant have previously been linked to white blood cell components (rs78440534), body mass index (rs1522687), bone density (rs3128952) and to body fat and reproductive hormone concentrations (rs11047908 and rs1888497).

Conclusion: This largest GWAS on *S. aureus* nasal carriage revealed novel genetic determinants differing by sex and carriage phenotype. Our ongoing research will replicate findings, perform meta-analysis, and explore genetic factors influencing the transition from carriage to infection using the DBDS, Copenhagen Hospital Biobank (>323,000 participants), and other international cohorts.

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P18.031.A Decoding syncope: genetic loci linked to sleep, psychiatric disorders, cardiovascular and autonomic dysregulation

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Background: Syncope and collapse (i.e. fainting) is a transient loss of consciousness, usually caused by reduced cerebral perfusion due to vasodilatation. Syncope is common, more frequent in females with a lifetime prevalence of 35% but the understanding of its genetic background remain incomplete.

Material and Methods: We performed a new genome-wide association study (GWAS) meta-analysis for syncope and collapse across four European cohorts (FinnGen, VA Million Veteran Program, Estonian BioBank, DeCODE Genetics), encompassing a total of 1,545,493 participants.

Results: We identified 75 genomic risk loci ($P < 5.0 \times 10^{-8}$), of which 57 were not previously reported, mapping near 487 different genes. The strongest association was observed on chromosome 2, with the lead intergenic variant rs12465214 ($P = 3.45 \times 10^{-75}$) near *ZNF804A*, the risk gene for schizophrenia, previously reported as implicated in syncope risk. New associations were discovered: e.g., a locus was identified near *PAX8* (rs62158163, $P = 6.88 \times 10^{-10}$), a gene involved in circadian rhythm and sleep duration. Functional analyses reported that several loci mapped to genes involved in sleep regulation (e.g., insomnia, nightmares, long sleep), psychiatric traits (e.g., mood instability, smoking, neuroticism), and autonomic dysregulation. Genetic correlation results also showed a shared genetic background between syncope and these traits. Stratified linkage disequilibrium score regression (sLDSC) also suggested which

brain regions and cell types may be implicated in syncope through genetic regulation mechanisms, revealing a multifaceted pathway underlying the genetic architecture of syncope.

Conclusion: This study highlights the previously reported complex genetic architecture of syncope, and reveals novel promising associations with sleep, psychiatric, and autonomic-related traits for further investigation.

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P18.032.C GWAS of in infant and early childhood height identifies 179 loci and reveals three cluster with different biology

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Background: We have limited knowledge on height genetics in childhood and its potential relationships with health outcomes. We characterized the genetics of height from birth to 8 years of age.

Material and Methods: GWAS was performed on height at 12 time points in the Norwegian MoBa study, with sample sizes ranging from 72473 (birth) to 27813 (8 years). Significant SNP effect sizes across time were subjected to hierarchical clustering and compared with self-recalled height at age 10 and adulthood UKBB GWAS. GLAD4U Enrichment analysis and genetic correlations were performed.

Results: We identified 179 independent SNPs across all time points and obtained three clusters: Cluster 1; contains SNPs with their strongest effects during infancy, Cluster 2; includes SNPs with strong effects from 1 to 8 years of age and weaker in adulthood. Cluster 3; consists of SNPs with constant effects starting from 1 year old, which increase in adulthood. Enrichment based on Clusters 1 and 2 SNPs showed traits related to glucose homeostasis. We identified 19 signals not associated with adult height. All belonged to clusters 1 and 2. Some of these SNPs were nearby genes such as *UBE2E* (rs822762) and *SELL*(rs6663862), related to type 2 diabetes, and *AKR1C1*(rs2904799), related to progesterone metabolism. Length at birth was genetically correlated ($p < 0.05$) with fasting insulin ($rg = -0.18$) and HOMA-IR ($rg = -0.19$). Height at 7 years was correlated ($rg = 0.12$) with T2D and preeclampsia ($rg = 0.18$).

Conclusion: We show that childhood height has a time dependent dynamic genetic basis and close links to metabolic related traits.

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P18.033.D The refinement of the priority index pipeline reveals known ALS biological pathways and suggests novel drug targets

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Background: Previously, we adapted the priority index pipeline (Pi) by Fang et al. (2019) to identify potential genes associated with Amyotrophic Lateral Sclerosis (ALS). Our recent work involved refining the pipeline by optimizing input data, integrating genes from pathway enrichment analysis, and adjusting internal parameters to enhance its capabilities.

Material and Methods: A GWAS summary statistic exclusively from European samples [van Rheenen et al., 2021] and top genes identified through an enrichment pathway analysis using Pascal [Lamparter et al., 2016] were utilised to choose input variants for the pipeline. The prioritisation of genes involved integrating various predictors, including gene proximity, chromatin interactions, eQTL associations, ontology connections, and network associations through Fisher's combined meta-analysis. The combined p-values were recalibrated to create a Pi rating scale, ranging from 0 to 5.

Results: Initially, 1,382 variants meeting FDR adjusted $P <= 0.05$ criteria were picked from an ALS GWAS that exclusively utilised European samples. Pascal analysis using the GWAS summary statistics unveiled 320 ALS-related genes. A 50k bp gene region identified 21,152 variants after eliminating duplicates from the initial selection which are associated with 756 genes through proximity, chromatin interactions in brain cells, and their impact on spinal cord and brain cortex expression. Using the combined information, Pi pipeline produced a ranked list of 5,694 ALS-linked genes.

Conclusion: The top identified genes show enrichment in ALS-related pathways, particularly in autophagy. Optimizing input data using both GWAS and pathway information improved the ranking of established ALS genes and provided potential novel targets.

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P18.034.A Biological age acceleration is more prevalent in men than in women at time of spontaneous intracerebral hemorrhage and ischemic stroke

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Background: Spontaneous intracerebral hemorrhage (ICH) occurs later in life in women compared to men. Although previous studies have demonstrated epigenetic age acceleration (EAA) differences in ischemic stroke (IS) patients - a measure of an individual's biological aging -it remains unknown whether this sex dimorphism also applies to ICH patients

Methods: We joined two ICH cohorts ($N = 200$, 45% women: 76.8 ± 13 years; 55% men: 67.9 ± 14 years). DNAm levels were obtained from whole blood samples using Illumina's EPIC array.

We evaluated three age-predictor clocks (Horvath, Hannum and Zhang.BLUP) and one health-status clock (Levine) and their respective EAA metrics including extrinsic EAA (EEAA) and intrinsic EAA (IEAA) measures. We compared aging measures between women and men, then performed an ICH-location stratification and interaction analysis followed by specificity analyses evaluating sex differences in non-stroke samples (N = 350) a new IS cohort (N = 657) and a sex-stroke phenotype interaction study.

Results: Women at time of ICH present lower EAA values than men (Horvath-EAA, p-value $= 1 \times 10^{-04}$; Hannum-EAA, p-value $= 8.1 \times 10^{-06}$; Zhang.BLUP-EAA, p-value $= 4.4 \times 10^{-04}$) as well as lower extrinsic IEAA values (Horvath-IEAA, p-value $= 3.1 \times 10^{-03}$; Hannum-IEAA, p-value $= 6.5 \times 10^{-04}$). We observed a sex-ICH location interaction (Zhang.BLUP-EAA, p-value $= 6.8 \times 10^{-02}$) apparently driven by differences in deep-ICH patients. Likewise, a sex-stroke phenotype interaction was observed, were non-ICH females have lower acceleration values (Hannum-EAA: −10,37) than non-ICH men (Hannum-EAA: −8,01), but the differences are smaller than in ICH cases (ICH-women Hannum-EAA: −9,26, ICH-men Hannum-EAA: −2,6)

Conclusion: Women show lower epigenetic age acceleration than men after ICH, driven by deep-ICH, with weaker but similar patterns in IS patients and non-stroke controls.

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P18.035.B Genome of Europe pilot studies: stepping-stones towards a pan-European reference database

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Background: Within the 1 million genomes (1+MG) initiative, a digital Europe (DEP)-funded project called the Genome of Europe (GoE) will collect whole-genome sequencing data from >100,000 European citizens across 51 contributing partners from 29 countries. GoE aims to improve and disseminate understanding of the genetic dimension to public health in Europe, through interactions with the European Rare Disease Research Alliance (ERDERA), the European Genomic Data Infrastructure (GDI), and the upcoming Personalised Cancer Medicine (PCM) Joint Action.

Methods: The GoE analyses will operate in a federated environment established by the GDI project, which raises technological and methodological challenges. Here we provide progress updates on the six use cases of GoE, following the beginning of the project in October 2024.

Results: To inform aggregation of individual-level data, we have begun pilot studies to determine methodology for describing population structure in independent datasets, through techniques such as federated principal-component analyses. Functionalities for variant frequency look-up tools have been evaluated, including assessment of the potential impact of heterogeneity in sequencing technology and bioinformatic pipelines. We also assess possible approaches for federated imputation algorithms and perspectives for generating polygenic (risk) score distributions, calibrated for ancestry. GoE will place particular attention on clinically-relevant variant frequencies in actionable-disease and pharmacogenetic genes. Structural variation, potentially of high impact, will also be investigated in GoE via long-read sequencing for >5,000 individuals.

Conclusion: Progress has begun to leverage the prospective >100,000 whole genome-sequences of GoE for public health genomics; with an outlined roadmap for alignment with other key European initiatives.

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P18.036.C GWAS meta-analysis identifies five novel susceptibility loci for endometrial cancer

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Background: Endometrial cancer is the most common gynaecological cancer in developed countries. In addition to environmental risk factors, genetic predisposition contributes towards endometrial cancer development but is still incompletely defined.

Methods: Building on genome-wide association studies (GWAS) by the Endometrial Cancer Association Consortium, we conducted a GWAS meta-analysis of 17,278 endometrial cancer cases and 289,180 controls, incorporating biobank samples from the UK, Finland, Estonia and Japan.

Results: GWAS analysis identified five novel risk loci (3p25.2, 3q25.2, 6q22.31, 12q21.2, and 17q24.2). Corresponding gene-based analyses supported findings for three of the novel loci, at *NAV3* (12q21.2), *PPARG* (3p25.2), and *BPTF* (17q24.2), as well as two additional candidate risk regions at *ATF7IP2* (16p13.2-p13.13) and *RPP21* (6p22.1). Validation genotyping in further independent case-control series replicated the most significant locus at 12q21.2, and functional genomic data suggested *NAV3* as a candidate causal gene. Silencing *NAV3* in endometrial cell lines accelerated cell division and wound healing capacity whereas NAV3 over-expression reduced cell survival and increased cell death, indicating that NAV3 acts as a tumour suppressor in endometrial cells.

Conclusion: Our large study extends the number of genomic risk loci identified for endometrial carcinoma by one-third and proposes a novel role of *NAV3* in the aetiology of this common cancer.

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P18.037.D Mitochondrial heteroplasmy is associated with disease manifestation in *LRRK2*-related Parkinson's disease

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Background: Mitochondrial heteroplasmy has been implicated in predicting disease status in monogenic *PRKN/PINK*-related Parkinson's disease (PD). While mitochondrial dysfunction and mitochondrial DNA (mtDNA) abnormalities have also been reported in *LRRK2*-associated PD (*LRRK2*-PD), the relationship between heteroplasmy and PD risk among *LRRK2* variant carries has not been thoroughly explored yet.

Methods: We assessed *LRRK2* variant carriers from our in-house cohort, which was complemented by the analysis of N = 2669 individuals from the Parkinson's Progression Markers Initiative. In total, we include N = 461 *LRRK2* variant carriers (affected: N = 230, unaffected: N = 231, pathogenic *LRRK2* variants: p.Gly2019Ser and Arg1441Gly). Blood-derived DNA underwent long-range PCR of the complete mitochondrial genome, followed by deep Nanopore long-read sequencing. Heteroplasmy was identified using *Mutserve2* variant caller. The association with affection status was assessed with a multiple logistic regression model, adjusting for age. The correlation between age and heteroplasmic variant load was assessed using the Spearman correlation.

Results: A higher mitochondrial heteroplasmic variant load was associated with PD affection status in *LRRK2* variant carriers ($\beta = 0.32$, $p = 0.005$). Notably, heteroplasmy correlated with age in affected carriers (rho = 0.16, $p = 0.016$) but not in unaffected carriers. As a future perspective, heteroplasmy in *LRRK2*-PD can be investigated utilizing the genetic data available within the Global Parkinson's genetic program.

Conclusion: Our findings reveal an accumulation of mtDNA heteroplasmy in affected *LRRK2* variant carriers. These results suggest that mitochondrial heteroplasmy could serve as a blood-based biomarker for predicting *LRRK2*-PD risk.

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P18.038.A Sex-specific effects of prenatal alcohol exposure on DNA methylation and gene expression in human placenta

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Background/Objectives: Prenatal alcohol exposure (PAE) is one of the most common causes of birth defects and some of the PAE-associated phenotypes are known to be sex-specific. However, the molecular mechanisms causing these differences are still largely unknown. Here, for the first time, we study the sex-specific effects of PAE on genome-wide DNA methylation (DNAm) and gene expression in human placenta.

Methods: We studied placental samples from our epiFASD cohort of PAE (n = 87), smoking-exposed (n = 11), and control newborns (n = 77). Genome-wide DNAm analyses were performed with Infinium MethylationEPIC microarray (Illumina). Data was normalized with InterpolatedXY method, which normalizes both autosomal and sex chromosome probes without introducing artificial sex bias to the data, cell-type adjusted with CIBERSORT method, and analyzed with limma R package. Gene expression was studied with 3'mRNA sequencing and analyzed with DESeq2 R package. We also analyzed differentially methylated regions and conducted pathways analyses.

Results: PAE-associated DNAm changes were highly sex-specific, and many genes with DNAm changes are linked to PAE phenotypes and have developmentally important functions. Our results revealed male-specific hypomethylation in TACR3, our previously identified PAE candidate gene. Interestingly, we found more PAE-associated placental gene expression changes in males (201 genes with FDR < 0.05) compared to females (23 genes with FDR < 0.05).

Conclusion: We discovered clear and highly interesting sex-specific differences in DNA methylation and gene expression, which should be considered in future studies of PAE. Furthermore, these findings help to define the etiology of sex-specific phenotypes of PAE.

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P18.040.C Unravelling the shared genetics and biological mechanisms of ADHD and obesity

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Background: Attention deficit hyperactivity disorder (ADHD) affects approximately 5% of children and 2.5% of adults, and is characterized by inattentiveness, impulsivity, and hyperactivity. Adults with ADHD have a 70% higher risk of developing obesity compared to individuals without ADHD, elevating cardiometabolic disease risk. This project aims to uncover the shared genetic architecture of ADHD and obesity through cross-disorder genome-wide analyses.

Material and Methods: We performed a large-scale cross-phenotype genome-wide association study (GWAS) of ADHD (38,691 individuals with ADHD and 186,843 controls) and body mass index (BMI) (N = 1.6M, serving as a proxy for obesity) in European population. Variants with concordant direction of effect on the two phenotypes were identified. The gene-based associations were calculated using MAGMA, and for genome-wide significant loci sets of credible variants were identified using

FINEMAP and linked to genes by integration with functional genomics data implemented in FLAMES. Subsequently, enrichment analyses were performed to identify affected tissues, brain developmental stages and cell types. Identified concordant variants will be used as basis for ADHD-BMI polygenic score (PGS) analyses in UK Biobank testing for phenome-wide association analyses.

Results: Preliminary findings revealed 206 genome-wide significant loci with a posterior probability of both phenotypes contributing greater than 0.9, involving 612 genes. The evaluation of the ADHD-BMI PRS in UK Biobank and inferred shared biological causes of ADHD and obesity concordant variants will be presented during the conference.

Conclusion: This study offers a foundation for understanding shared biological pathways of ADHD and obesity and informing future research and risk assessment strategies.

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P18.041.D Genetics of disease susceptibility and age of diagnosis for psychiatric disorders are surprisingly independent

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Background: Early onset of psychiatric disorders is linked to increased clinical severity and poorer outcomes. However, the strength of this association and the extent driven by genetics is poorly understood. Most genome-wide association studies (GWAS) have prioritized understanding disease susceptibility, ignoring age-at-diagnosis (AAD) information, or including it as a covariate despite evidence that it is heritable. This study examines the genetics of AAD and whether early diagnosis reflects higher genetic burden.

Material and Methods: Using iPSYCH data, we conducted GWAS for case-control status and AAD for ADHD, autism spectrum disorder (ASD), schizophrenia (SCZ), and major depressive disorder (MDD). LD Score Regression was used to estimate heritabilities for AAD and disease susceptibility, and their genetic correlations. We validated theoretical expectations by simulating AAD and case-control status under time-to-event models.

Results: We found that heritability of AAD is disorder-specific, with estimates of 0.15 (0.02) for ADHD, 0.11 (0.02) for ASD, and 0.03 (0.01) for MDD. We also identified two genome-wide significant variants for AAD of ADHD and one for AAD of SCZ. We estimated the genetic correlations between AAD and disease susceptibility, and found them to be surprisingly close to zero, in particular for ADHD, −0.014 (0.059), and ASD −0.139 (0.095).

Conclusion: AAD is a critical yet often overlooked trait in psychiatric genetics. Time-to-event GWAS approaches can improve understanding of genetic influences across the course of disease but require validation. Our findings suggest that genetic factors influencing disease onset may differ from those driving susceptibility, implying distinct genetic subtypes based on early versus late onset.

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P18.042.A LEAP/InovAND - a multiscale resource to explore genetics, brain imaging and clinical data in autism

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Background: We present a new resource for autism research combining quantitative phenotypic data, whole-genome sequencing (WGS), and brain structural and functional data (MRI, EEG). Participants include autistic individuals with or without intellectual developmental disability (IDD), relatives and typically developed (TD) individuals from the EUAIMS/AIMS2-Trials-LEAP and InovAND cohorts.

Material and Methods: Among 5,198 participants (39.23% females, mean age 18.56), 2,025 had a diagnosis of autism (>70% without IDD), 67 had a diagnosis of IDD, 1,803 are non-autistic relatives, and 1,303 are TD. WGS data is available for 2,720 individuals: 840 with autism, 37 with IDD, 1,118 non-autistic relatives, and 592 TD. For a subset of 346 individuals (172 autistic, 158 non-autistic) data across all modalities is available (WGS, MRI, EEG, clinical).

Results: Using IQ, the short sensory profile, and Vineland II data, autistic and non-autistic individuals were stratified into six clusters: Cluster 1 includes 75% of TD individuals, Cluster 2 and 3 includes autistic individuals with IDD. Cluster 2 is enriched in de novo variants falling in High Confidence NDD genes and in low polygenic scores for intelligence. Clusters 3, 4 and 5 contain individuals with high polygenic scores for autism. Cluster 3 shows an increased temporal cortex thickness. Interestingly, Cluster 6, including individuals with high intensity of sensory problems, does not show a specific genetic and brain profile.

Conclusion: This resource supports researchers integrating multiscale data to explore links between genetic variations, brain

anatomy/function, and autism's phenotypic diversity. A webpage will share project details and results obtained with this resource.

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P18.043.B Central role of glycosylation processes in human genetic susceptibility to SARS-CoV-2 infections with Omicron variants

Frank Geller[1], *Xiaoping Wu*[1], *Vilma Lammi*[2], *Erik Abner*[3], *Jesse Valliere*[4], *Katerina Nastou*[1], *Ole Pedersen*[5], *Jacqueline Lane*[4], *Ria Lassaunière*[1], *Hanna Ollila*[6], *Sisse Ostrowski*[7], *Bjarke Feenstra*[1]

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Background: The genetics of SARS-CoV-2 infection has been investigated by the Host Genetics Initiative in about 220,000 cases diagnosed before the predominance of the Omicron variant in late 2021. We performed a genome-wide association study of SARS-CoV-2 infection with Omicron variants including more than 150,000 cases and more than 550,000 controls with no known SARS-CoV-2 infection from four cohorts.

Material and Methods: Cases of SARS-CoV-2 infection with Omicron and controls were identified from electronic health records in Denmark, Finland, Estonia and the US.

Results: We identified 13 genome-wide significant loci, of which only five were previously described as associated with SARS-CoV-2 infection. The strongest signal was a SNP intronic of *ST6GAL1*, a gene affecting immune development and function, and connected to three other associated loci (harboring *MUC1*, *MUC5AC* and *MUC16*) through O-glycan biosynthesis. We also found further evidence for an involvement of blood group systems in SARS-CoV-2 infection, as we observed association 1) for a different lead SNP at the *ABO* locus indicating a protective effect of blood group B against Omicron infection, 2) for the *FUT2* SNP tagging secretor status also reported for SARS-CoV-2 infection with earlier variants, and 3) for the strongest protein quantitative trait locus for *FUT3* (Lewis gene).

Conclusion: Our study shows that the human genetic architecture of SARS-CoV-2 infection is under constant development. The results provide robust evidence for individual genetic variation related to glycosylation translating into susceptibility to SARS-CoV-2 infections with Omicron variants.

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P18.044.C Investigating the use of Lysosomal PRS to estimate Parkinson's Disease risk

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Background: Parkinson's disease (PD) is a complex disorder resulting from the interplay of environmental and genetic factors. As only 5–10% of cases can be explained by monogenic forms, a polygenic additive effect has been proposed to explain the missing heritability. Considering that several PD genes are involved in the dysregulation of lysosomal clearance (i.e., LRRK2, VPS35 and GBA1), promoting pathological alpha-synuclein aggregation, we constructed a lysosomal pathway PRS to establish its contribution on PD risk.

Material and Methods: Using the latest European PD-GWAS summary statistics, we extracted all SNPs encompassing a manually curated list of lysosomal pathway genes. Lysosomal-PRS were created for the European COURAGE-PD cohorts not included in the base dataset exploiting PRSice and PRSet, using our largest cohort for model's training. A meta-analysis of all cohorts was performed using metafor.

Results: We selected a total of 980 lysosomal genes after querying KEGG, Gene Ontology and GSEA databases and extracted 367.626 SNPs from the PD-GWAS summary statistics. After performing individual-cohort QC following our established pipeline, we carried out the analysis on 23 European sites (average N = 649). Our training set comprised 2739 individuals (PD = 51%). PRSice C+T was used to select the best model fitting the training set, obtaining 58 significant SNPs (model p-val: 3.16E-20). Using this model, we obtained 4% improvement in correctly classifying patients with PD (PRS model: AUC = 0.68; null model: AUC = 0.64).

Conclusion: Our study highlighted lysosomal-PRS showed better predictive power for PD.

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P18.045.D Whole-genome sequence analysis of 450'000 individuals across diverse populations identifies new rare and low-frequency colorectal cancer risk variants

Aurélie Kamoun[1], *Liza Darrous*[1], *Simon Leyss*[1], *Gareth Hawkes*[2], *Robin Beaumont*[2], *Harry Wright*[2], *Michael Weedon*[2], *Lauric Ferrat*[1], *Timothy Frayling*[1]

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Background: Genome-wide association studies have primarily identified common variants with small effects. To uncover rarer variants with larger effects on colorectal cancer (CRC) risk, where excess adiposity has a strong causal role, we meta-analysed 70 million variants, in 450'000 individuals with whole-genome sequencing (WGS) data from All of Us (AOU) and UK Biobank (UKB).

Material and Methods: We performed extensive QC at the sample, variant, and genotype levels. We selected cases using either a strict (N = 11'402) or a broad (N = 37'110) definition, the latter including benign tumours, and kept as controls all participants with no history of cancer (N = 409'937). The selection was based on available ICD9/10 and SNOMED codes for AOU, and ICD9/10, READ2 and CTV3 codes for UKB. We validated our phenotype definitions using polygenic risk scores. Pan-ancestry analyses in AOU were conducted using REGENIE, adjusting for 16 population PCs. Additional analyses were run in UKB and AOU individuals with ≥80% predicted European ancestry, adjusting for 20 population PCs.

Results: From the broad case definition, we identified 27 variants at Bonferroni significance ($p < 7e\text{-}10$) with a MAF below 0.05 spanning 5 distance independent loci, including 24 novel risk variants for CRC, most of them in or near genes involved in the disease onset or progression (*ASXL1*, *EIF3H*, *TP53*, *CHRDL2*, *ARHGAP11A-SCG5*). The rarest variant (MAF = 0.0003) was identified in both pan-ancestry and European only analyses and reached an odd-ratio of 3.

Conclusion: Our findings highlight the potential of population-based WGS to identify rarer germline variations for colorectal cancer risk.

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P18.046.A Cross-ancestry meta-GWAS identified novel loci and causal pathways for carotid intima media-thickness in over 157,261 participants

Devendra Meena[1], *Jingxian Huang*[1], *Jian Huang*[2], *Ioanna Tzoulaki*[1], *Abbas Dehghan*[1], *The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE)*[3], *The University College London Edinburgh Bristol (UCLEB)*[3], *The UK Biobank*[3]

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Background: Atherosclerotic cardiovascular disease (CVD) remains the leading cause of morbidity and mortality, impacting over 523 million people worldwide. Carotid atherosclerosis, measured by carotid intima-media thickness (cIMT), is a strong predictor of future CVD events, including stroke. Genome-wide association studies (GWAS) have identified over 42 loci associated with cIMT, however, individuals from non-European populations remain underrepresented.

Material and Methods: We performed a large-scale cross-ancestry meta-GWAS of cIMT, encompassing up to 157,261 participants from five population groups of diverse ancestries: European, East Asian, African, admixed Latin Americans (Brazilian, and Hispanic). The meta-GWAS was performed using a fixed-effects inverse-variance-weighted model and meta-regression of multi-ethnic genetic association (MR-MEGA).

Results: Our analysis identified 61 loci, of which 33 are novel. Cross-ancestry fine-mapping identified 12 loci with less than five variants in 95% credible sets, highlighting potential causal variants. Mendelian randomisation suggested a potential causal association between cIMT and large artery stroke, but not for small-vessel stroke. Post-GWAS analyses, including transcriptome-wide association studies, further supported the GWAS findings by offering insights into the pathways underlying the identified loci. Finally, by integrating multi-omics data from publicly available datasets, we prioritized up to 20 plausible causal candidate genes and pathways involved in atherosclerosis, prioritizing genes and pathways for in vitro and in vivo validation. These findings may inform improved risk prediction and novel therapeutic strategies for atherosclerotic cardiovascular disease.

Conclusion: Our study is the first cross-ancestry GWAS for cIMT, paving the way for future efforts to identify cIMT loci in non-European populations.

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P18.047.B Functional dissection of 15q15.1 GWAS risk variants implicates *CHST14* in endometrial cancer development

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Background: The largest published endometrial cancer GWAS identified a credible set of 47 risk variants located in non-coding regions at the 15q15.1 locus. Previously observed enrichment of endometrial cancer GWAS variation in enhancer/promoter chromatin loops suggests these variants exert their effects through related gene regulation.

Material and Methods: 15q15.1 risk variants were mapped to putative enhancers, and target promoters identified using H3K27Ac HiChIP chromatin looping analysis in Ishikawa endometrial cancer cells. CRISPR interference validated enhancer activity and target gene regulation. Reporter gene assays tested the effect of risk variants on promoter activity. Colony formation, growth in low attachment and sulfated glycosaminoglycan assays were performed in Ishikawa and SPEC2 endometrial cancer cells following gene knockdown using siRNA or LNA GapmeRs.

Results: We identified eight risk variants in five putative enhancers at 15q15.1. Enhancer/promoter looping data implicated 13 target genes with robust expression in endometrial cancer cells. CRISPR interference validated enhancer activity and identified four target genes, including CHST14 and PAK6. Risk alleles in an enhancer targeting CHST14 and PAK6 increased promoter activity by ~2-fold. Knockdown of CHST14, encoding the enzyme responsible for sulfating the glycosaminoglycan dermatan, impaired cancer cell phenotypes and reduced sulfated glycosaminoglycan levels in two cell lines.

Conclusion: These findings identify CHST14 as a novel endometrial cancer susceptibility gene that may mediate its effects through increased production of sulfated glycosaminoglycan, a key constituent of the extracellular matrix.

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P18.048.C The common and rare facets of germline polygenicity converge through intercellular crosstalk in the tumor microenvironment of nasopharyngeal carcinoma

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Background: Germline genetic variations contribute to risk of nasopharyngeal carcinoma (NPC). Tumor microenvironment (TME) plays a crucial role in tumorigenesis. However, whether NPC-risk-associated genes express span multiple types within TME and

whether cell types enriched for genetic associations interact with each other are largely unknown.

Material and Methods: We integrate the genetic association data concerning both common or rare variants for NPC (N = 9645) and transcriptomic data of NPC tumors tissues (single-cell RNA **sequencing**: N = 31, bulk RNA **sequencing**: N = 180, spatial transcriptome: N = 7), conducting single cell level genetic association and cell-cell interaction analysis to uncover the contribution of NPC genetic susceptibility to the intercellular communication within the TME.

Results: We find that common and rare genetic association in NPC are enriched in antigen-presenting cells and tumor epithelial/stromal cells, respectively. Notably, such cell types of strong genetic association also exhibit enhanced interactions and closer spatial proximity than the cell types depleted for genetic associations. In particular, strong crosstalk is detected between the cell type with the strongest common genetic association (Langerhans dendritic cells) and the cell type with the strongest rare genetic association (an epithelial cell subtype marked by HILPDA). Clinically, joint consideration of the tumor infiltration levels of the two genetically-associated and inter-connected subtypes substantially improves the prediction for NPC prognosis.

Conclusion: We bring together genetic associations of both common and rare variants with multi-OMIC data of tumors, uncovering significant crosstalk between genetic-association-enriched cell types within the NPC TME. Understanding the genetically-influenced TME interactions substantially improves the prediction of clinical prognosis.

C.O.I. Disclosure:

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P18.049.D Extracting longitudinal traits to uncover the genetic architecture of adolescent facial growth

Nina Claessens[1], *Seppe Goovaerts*[1], *Jay Devine*[1], *Damian JJ Farnell*[2], *Mary L. Marazita*[3], *John R. Shaffer*[3], *Mark D. Shriver*[4], *Noah Herrick*[3], *Susan Walsh*[5], *Seth M. Weinberg*[3], *Peter Claes*[1]

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Background: During adolescence, complex changes in hard and soft tissues impact facial appearance. While cross-sectional datasets are typically used to model average facial growth, they cannot track individual growth trajectories, leaving the genetics of postnatal facial growth largely unexplored. This is crucial for orthodontics and craniofacial surgery, where variation in timing and growth rate can impact outcomes.

Material and methods: We extracted dense 3D facial landmarks from magnetic resonance images in the longitudinal ABCD study (N = 916) at ages 10, 12, and 14. To focus on growth rather than shape, we subtracted each individual's average face from their image at each timepoint. We then used functional principal component analysis to extract the principal trajectories of facial growth. Scores on these trajectories were used in a multivariate genome-wide association study. To boost genomic discovery, we leveraged pleiotropy with bone density and height at puberty onset using a conditional false discovery rate.

Results: The principal trajectories described both global and local changes and distinguished spatial (width versus lengthwise) and temporal (early versus late) growth differences. We identified two independent genomic loci (P < 5e-8) and 30 additional loci (5% FDR) by leveraging auxiliary traits. This includes one near *HMGA2*, a known growth-related gene, and another near *TTC5*, mutations in which cause global developmental delay and dysmorphic facial features.

Conclusion: We demonstrate how facial growth can be modeled from longitudinal imaging data and used to uncover genetic factors for facial growth. This work lays the foundation for future morphometric and genetic studies on individual facial growth.

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P18.050.A rHDL Uncovers Tissue-Specific Pleiotropy Landscape in Plasma and Serum Proteomes

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Background: Pleiotropy, where a single genetic variant influences multiple traits, is key to understanding complex diseases. Yet, scalable methods to quantify pleiotropy across proteomic datasets remain limited.

Material and Methods: We introduce rHDL (reversed high-definition likelihood), a maximum likelihood framework that estimates SNP-level pleiotropy by modeling the joint distribution of genome-association summary statistics across thousands of proteins. We applied rHDL to harmonize summary statistics of 1,350 proteins from two large-scale studies: plasma proteins (N = 35,559) and serum proteins (N = 5,368). Both were measured using SomaScan.

Results: rHDL identified 154 and 114 independent pleiotropic SNPs ($P < 5e-8$) for the plasma and serum proteomes. Among them, only 11 SNPs were overlapped, some of which were established pleiotropic loci, such as *CFH* and *ABO*. While the pleiotropic loci of the plasma proteome were enriched for complement cascade, the serum pleiotropic loci were enriched for coagulation pathways, e.g., the *VTN* locus exhibited the strongest pleiotropy in serum ($P < 1e-300$, 140 associated proteins). These differences suggest tissue-specific genetic influences on protein expression and pleiotropy, even when the same proteomic technology was used.

Conclusion: rHDL offers a robust method for evaluating pleiotropy across proteomes. The method's scalability supports future applications to multi-omics data, prioritizing SNPs for functional interrogation. Our findings highlight the importance of considering tissue context in genetic studies and provide new insights into the genetic architecture of complex diseases.

C.O.I. Disclosure:

R. Zhai: None.

P18.051.B The impact of genetics on different measures of metabolomic aging and common disease

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Background: We performed metabolomic profiling in a large biobank, and derived three metabolomic aging measures. We investigated their genetic determinants and interactions with genetic disease susceptibility.

Material and Methods: We measured 251 metabolomic parameters in UK Biobank blood samples using 1H-NMR and calculated three aging measures:

1. **Mortality Metabolomic Risk Age** indicates an individual's 10-year mortality risk. E.g. a risk age of 70 indicates the same predicted mortality as the average 70-year-old.
2. **Incident Disease Metabolomic Risk Age** reflects 10-year risk of 10 diseases with the largest loss of disability-adjusted life years.
3. **Metabolomic Clock Age** predicts chronological age from metabolomic parameters.

Models were trained on 245,009 and tested on 220,378 European ancestry individuals. GWAS was performed for 9.9M imputed variants using linear regression, adjusted for age, sex, and principal components. Interactions with polygenic risk scores (PGS) were assessed using Cox regression.

Results: Mortality and disease risk ages were highly correlated ($r = 0.906$) and moderately with clock age (r~0.45). Key metabolites for all scores included glycoprotein acetylation (an inflammation marker) and PUFA/MUFA ratio. Omega-3 was clock specific; LDL was disease/mortality specific.

241 loci were associated with metabolomic aging. 122 were shared across scores, including at *FADS2*, *HP* (haploglobin) and *APOE*. 76 were unique to mortality/disease, 9 to clock age. Mortality/disease ages showed negative interactions with diabetes and myocardial infarction PGS, indicating a weaker role for genetic risk in individuals with advanced metabolomic age.

Conclusion: Metabolomic profiling reveals shared and unique genetic drivers of healthy aging and modifies genetic disease risk.

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P18.053.D Multi-ancestry GWAS meta-analysis of stool frequency identifies key signatures of the physiology of gut (dys)motility and gastrointestinal disorders

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Background: Studying endophenotypes of gut (dys)motility may represent an effective strategy to identify its genetic determinants and characterize molecular pathways relevant to irritable bowel syndrome (IBS) and other gastrointestinal disorders.

Material and Methods: We performed a multi-ancestry genome-wide association study (GWAS) meta-analysis of stool frequency (number of bowel movements per week) in 268,606 individuals of European (EUR) and East Asian (EAS) ancestry from six population-based cohorts. Heritability and genetic correlations with other traits were estimated, followed by Mendelian Randomization to identify causal relationships across these. Association signals were functionally annotated and fine mapped, to prioritize candidate genes/variants and corresponding causative mechanisms.

Results: EAS and EUR populations showed similar SNP heritability (5.6% and 7.0%, respectively) and genetic architecture ($r_g = 0.88$). Genetic correlations with gastrointestinal and psychiatric disorders were confirmed (r_g range 0.18–0.47), and novel correlations with multiple cardiovascular traits (r_g range 0.12–0.14) were detected and highlighted by computational analysis of drug signatures. Altogether, 21 independent GWAS signals were detected, 18 across ethnicities and 3 EUR-specific. Three loci were fine mapped to a single causative variant (PIP range 0.74–0.93), involving genes relevant to glia (*ZFHX4-AS1*), neuronal function (*XPR1*) and development (*SLC35F3*). Strong candidates in other loci include genes functionally linked to bile acid synthesis (*KLB*), cholinergic transmission (*COLQ*), and metabolic disorders (*FTO* and *CDKAL1*).

Conclusion: Multi-ancestry GWAS meta-analyses of stool frequency resulted in the discovery of candidate genes and molecular pathways relevant to the physiology of gut motility across ethnicities. Several of these offer actionable targets towards therapeutic exploitation in IBS and other dysmotility syndromes.

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P18.054.A Impact of cell type-specific genetic variations in aortic distensibility

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Background: Aortic distensibility is the ability of arteries to expand with pulse pressure induced by the cardiac cycle, and it often diminishes with age. Previous GWA studies have identified SNPs linked to distensibility. Despite the established genetic associations, the mechanisms driving changes in distensibility remain unclear. This study aims to illuminate factors influencing distensibility using existing gene expression data, focusing on age-related changes in aortic cellular composition, alongside associated genetic variations and cell type-specific gene expression (CT-GE).

Methods: We applied a deep-learning model to segment 62,497 CMRI images from UKBB and calculated aortic distensibility. GWA was conducted on 56,765 participants using BOLT-LMM. We utilized CIBERSORTx to perform GE deconvolution on 432 GTEx participants with aortic GE data, analyzing changes in cell composition and CT-GE associated with age and GWA hits.

Results: Distensibility was calculated for 56,765 subjects. None of the 33 independent SNPs across 12 loci from the GWA showed an association with inferred cellular proportions, following multiple test correction. Pericytes and Fibroblast-I proportions declined with age, whereas VSMC-II tended to increase. CT-GE analysis revealed associations of GWA SNPs with *SRR* gene expression in VSMC-I, VSMC-II, and Fibroblast-I cells and with expression of *CDH13* in VSMC-II.

Conclusion: This study contributes to understanding of variation in aortic distensibility associated with age and genotype. Examining variation in aortic distensibility through the lens of gene expression in the relevant tissue suggested that variation in the expression of *SRR* in VSMC-II and Fibroblast-I, and *CDH13* in VSMC-II could contribute to the observed genetic associations.

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M. Chopra: None. **N. Hynes:** None. **C. Seoighe:** None.

P18.055.B Novel rare variants underlying adiposity-related traits: A whole-genome sequence analysis of 745,000 individuals across diverse populations

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Genome-wide association studies (GWAS) have largely uncovered common variants with modest effects on complex traits. To identify rarer variants with larger effects on adiposity-related traits (Body Mass Index, Waist-Hip Ratio adjusted for BMI, and Triglycerides), we examined 111 million variants observed in at least 20 of 745,000 individuals with whole-genome sequencing (WGS) data from the UK Biobank and All of Us studies, over 80% of which were rare ($0.001 > MAF > 0.0001$) or very rare ($MAF < 0.0001$).

In each study, we performed pan-ancestry analyses using an additive model in REGENIE, adjusting for 40 PCs in UK Biobank and 16 PCs in All of Us, and repeated analyses in individuals with ≥80% predicted European ancestry using 20 ancestry-specific PCs.

For BMI and WHRadjBMI, we discovered 2 and 3 independent rare or very rare variants respectively, at a conservative Bonferroni-corrected P-value (< 4.47–6.28e-10), after conditioning on variants from the largest published GWAS. For Triglycerides, we identified 7 such variants, of which 3 were located >500 kb from known variants.

Variants identified included those present in as few as 1 in 9,000 people. Examples included known and novel variants in or near INHBE and PLIN1 (WHRadjBMI), ASPSCR1 (BMI), and LIPE (Triglycerides), with effect sizes ranging from 0.13 to 0.78 standard deviations and consistent across pan-ancestry and European-only analyses.

Our findings highlight the role of rare variants in metabolic traits and demonstrate the growing potential of WGS in identifying rare, highly penetrant alleles, advancing our understanding of adiposity and lipid metabolism.

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P18.057.D Novel insights into handling of trace elements in urine via large-scale genetic screening

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Background: Trace elements (TEs) are vital for maintaining essential physiological functions but can also exhibit toxic effects. Previous genome-wide association studies (GWASs) have concentrated on genetic determinants of blood TE levels. Studies of the genetic architecture of urinary TE concentrations can provide complementary insights into TE regulation and metabolism, given the importance of the kidney in regulating TE levels.

Material and Methods: We measured 22 TEs by mass spectrometry (ICP-MS) from urine samples of up to 10142 individuals from three cohorts: GCKD ($N = 4870$), CoLaus ($N = 4541$), and SKIPOGH ($N = 731$). We performed a GWAS of every TE in each cohort ($N = 9.6$ million variants) and meta-analyzed the results using an inverse variance weighted method. We then employed genetic colocalization analysis and integrated published blood TE GWAS results to assess whether TE metabolism is driven by the same or distinct loci in urine and blood.

Results: Our meta-analysis identified four genome-wide significant loci ($p < 5e\text{-}8$) associated with urinary cadmium, selenium ($N = 2$), and chromium. Genetic variants associated with urinary cadmium at the XKR9/LACTB2 locus colocalized with blood cadmium levels ($PP.H4 = 0.68$), LACTB2 expression in blood ($PP.H4 = 0.7$, eQTLGen) and plasma LACT2B levels ($PP.H4 = 0.69$, UKB-PPP). We also discovered a novel association between urinary selenium and the INMT locus (encoding indolethylamine N-methyltransferase involved in selenium metabolism), not previously implicated in blood TE studies.

Conclusion: This first GWAS of 22 urinary TEs revealed shared and distinct genetic determinants of TE handling between blood and urine, highlighting the value of studying multiple biological biofluids to understand TE regulation in the human body.

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P18.058.A The genomic structure of normal and maladaptive personality in the Genetics Links to Anxiety and Depression sample using improved linkage disequilibrium score regression

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Background: Personality traits are dimensional phenotypic constructs correlating with most other behavioural traits. Typically, genetic correlations mirror phenotypic. The commensurability of personality across normal and maladaptive personality, frameworks employing different number of factors, and the genetic underpinnings of these, has been less investigated in larger samples.

Material and Methods: Subjects from the Genetic Links to Anxiety and Depression study and the United Kingdom National Institute of Health and Social Care Research BioResource were assessed for normal and extreme personality measurements using the HEXACO (N = 14,190) normal personality scale, the EPQ Neuroticism scale (N = 20,139), and the Personality PID-5 brief form (N = 20,144). Heritabilities and genetic correlations were obtained using an improved version of LD score regression (LDSR) and GCTA-GREML.

Results: The six HEXACO dimensions, 5 PID-5 factors, and EPQ Neuroticism had significant GCTA GREML heritabilities between 8–15%. Notable LDSR genetic correlations (rg) were seen for Honesty-Humility with Neuroticism [rg = −0.59 (0.3), p = 0.017] and Negative Affect [rg = −0.57 (0.26), p = 0.038]. Additionally, a strong genetic relationship between PTSD and extreme personality was seen. Personality dimensions where phenotypic and genetic correlations were consistent included Extraversion and Detachment, where for some other traits such as Openness and Psychoticism, genetic correlations did not reflect phenotypic.

Conclusion: Our findings highlight specific consistent and inconsistent relationships across phenotypic and genetic measurements for normal and maladaptive personality dimensions and psychiatric disorders, as well as the utility of a personality framework with six dimensions.

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P18.059.B Whole exome analysis identifies genetic variants contributing to serological antibody responses against pathogens

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Background: Genetic factors influence immune responses to infections, but the underlying mechanisms remain poorly understood. This study investigates the genetic determinants of serological antibody responses and their broader phenotypic associations.

Material and Methods: Whole exome sequencing data from 7,192 individuals in the UK Biobank was analysed to identify genetic variants contributing to variability in antibody responses against common pathogens. Association testing, fine-mapping, and gene burden analyses were employed to uncover causal loci influencing immune responses. A phenome-wide association study (PheWAS) was then conducted to examine the broader phenotypic effects of these genetic risk alleles.

Results: The study identified 31 exonic variants significantly associated with antibody responses, including loci within the major histocompatibility complex (MHC) region. Fine-mapping refined these associations to 30 putative causal variants. The PheWAS revealed 304 significant associations between 25 genetic variants and 98 clinical traits, with colocalization analyses identifying 36 instances of shared genetic effects across different phenotypes. These findings suggest that infection-related genetic variants also influence susceptibility to non-communicable diseases, revealing pleiotropic effects.

Conclusion: These results highlight the complex interplay between genetics and immune responses, demonstrating the utility of exome sequencing in identifying functionally relevant genetic variants. The study advances our understanding of host-pathogen interactions and lays the foundation for future personalized strategies in infectious disease prevention and treatment.

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Z. Zhu: None. **M. Inouye:** None.

P18.060.C Genome-wide association study of susceptibility to sepsis-associated acute respiratory distress syndrome

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Instituto de Investigación Sanitaria de Canarias, Las Palmas de Gran Canaria, Spain, [18]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada.

Background. Acute respiratory distress syndrome (ARDS) is a severe inflammatory lung disorder, mainly caused by sepsis, with a hospital mortality rate of around 40%. ARDS pathogenesis involves complex genetic and environmental interactions. This study aimed to identify novel genetic variants associated with post-sepsis ARDS to better understand its underlying biological mechanisms.

Materials and Methods: We conducted genome-wide association studies (GWAS) in three independent cohorts from Spain, Germany and United Kingdom. We meta-analysed the results to generate the largest meta-GWAS of sepsis-associated ARDS to date comprising 716 sepsis-associated ARDS cases and 4,399 sepsis controls. Variant associations were declared statistically significant if they had a meta-analysis $p < 5 \times 10\text{-}8$. Bayesian fine-mapping and in-silico functional assessments were performed, along with rare variant collapsing analyses using whole exome sequencing (WES) data from a patient subset (260 cases and 537 controls). Validation of results was sought in data from 430 sepsis-associated ARDS cases and 1,398 controls with sepsis from two other independent studies.

Results: We identified a novel genome-wide significant association with post-sepsis ARDS ($p = 3.43 \times 10\text{-}8$) intergenic to *ANKRD31* and *HMGCR* and previously linked to lipid and cholesterol metabolism. While it did not reach nominal significance in the two additional validation cohorts, it had consistent direction of effects across all 5 studies. Gene-based rare exonic variant analysis results would also suggest the role of this locus on ARDS.

Conclusion: A common variant between *ANKRD31* and *HMGCR* is associated with post-sepsis ARDS risk, implicating lipid metabolism in ARDS pathophysiology. Validation in independent studies is needed.

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P18.061.D Shared genetics across fibrotic traits

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Background: Shared pathophysiologic features of fibrotic traits, irrespective of location-of-onset, have been suggested across various fibrotic traits. However, the degree and specificity of shared genetic underpinnings across fibrotic traits remain poorly understood.

Methods: We investigated the genetic architecture of 13 fibrotic traits, including both organ (e.g., cirrhosis, idiopathic pulmonary fibrosis, and myocardial fibrosis) and non-organ fibrotic traits (e.g., Dupuytren's contracture, trigger finger and Peyronie's disease). We conducted genome-wide meta-analyses for eight fibrotic traits and queried publicly available data for an additional five fibrotic traits. Global genetic correlations were assessed using Linkage Disequilibrium Score Regression, and locus-specific genetic overlap was evaluated through pairwise colocalization analyses.

Results: Across all 13 fibrotic traits, we identified 342 genome-wide significant variants, of which 108 were novel. The most significant risk variant was for Dupuytren's contracture in *WNT7B* (known rs28971325, MAF = 23%, $\beta = 0.85$, $P = 6.8 \times 10^{-1384}$). The strongest global genetic correlation was observed between carpal tunnel syndrome and trigger finger ($r_g = 0.59$, $P = 4.0 \times 10^{-78}$). Notably, four risk variants reached genome-wide significance across three or more fibrotic traits, including rs28971325 (posterior probability $4 = 0.99$), which was associated with Dupuytren's contracture, Peyronie's disease, and plantar fibromatosis, and rs11205303 near MTMR11/OTUD7B, which was implicated in carpal tunnel syndrome, Dupuytren's contracture, and Peyronie's disease.

Conclusion: Our findings demonstrate a shared genetic architecture across multiple fibrotic traits, revealing key overlapping biological pathways while also identifying distinct genetic signals for specific fibrotic conditions.

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P18.062.A Glycemic vs. Erythrocytic Genetic Influences on HbA1c: Insights from an East Asian Cohort

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Background: Glycated hemoglobin (HbA1c) is vital for diagnosing type 2 diabetes (T2D) and monitoring long-term glycemic control. Although previous genome-wide association studies (GWAS) have uncovered single nucleotide polymorphisms (SNPs) associated with HbA1c, the distinction between glycemic- and erythrocyte-driven genetic influences remains underexplored, particularly in East Asian populations.

Material and Methods: We retrieved 45 top-ranking SNPs associated with HbA1c from the Korean Genome and Epidemiology Study (KNIH PheWeb GWAS, N = 51,385). To characterize their mechanistic pathways, we performed linear regressions in a Korean cohort (N = 2,116), adjusting for principal components 1–10, sex, and age. Associations between these SNPs and three phenotypic domains—HbA1c levels, glycemic traits (e.g., fasting glucose), and erythrocytic traits (e.g., hemoglobin, hematocrit)—were tested. Variants were classified as "glycemic" or "erythrocytic" if they significantly overlapped ($P < 0.05$) with relevant traits in addition to HbA1c. SNPs with trait-specific (but not HbA1c) associations were categorized as candidate variants.

Results: Among 16 HbA1c-associated SNPs ($P < 0.05$), nine (e.g., rs1799884, rs340515) showed glycemic-related associations, while two (rs59616136, rs2305196) were linked to erythrocytic measures. We also discovered 11 glycemic and six erythrocytic candidate SNPs that did not coincide with HbA1c signals. Notably, certain erythrocytic variants may alter red blood cell dynamics, leading to HbA1c values that do not accurately represent glycemic status in some individuals.

Conclusion: By delineating glycemic- versus erythrocytic-based genetic influences, these findings highlight the need for genotype-informed interpretation of HbA1c, potentially refining T2D diagnosis and personalized diabetes care.

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P18.063.B Genetic and Prognostic Insights from Deep Learning Segmentation of Carotid Lumen Diameter

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Background: Carotid ultrasound is a non-invasive imaging modality widely used in clinical practice to assess vascular anatomy and function. A key application is measuring carotid Intima-Media Thickness (cIMT), an important marker of atherosclerotic burden in the common carotid arteries (CCAs). Emerging research indicates that increased cIMT may lead to a compensatory enlargement of the lumen diameter (cLD) in CCAs. However, the genetic determinants of cLD, its association with other cardiovascular traits, and its prognostic value remain unexplored.

Material and Methods: To investigate these questions, we trained a Deep Learning model to automatically segment the carotid artery from ultrasound images, enabling the extraction of cLD measurements in CCAs. We applied genome-wide association studies (GWAS) to examine the genetic basis of cLD and its relationship with cIMT using a cohort of 18 804 individuals from the UK Biobank. Furthermore, we investigated cLD's prognostic power by running a survival analysis to assess its association with cardiovascular events.

Results: We found that cLD and cIMT exhibit distinct heritability estimates, indicating different genetic and environmental influences. Additionally, we investigated the predictive power of cLD and cIMT and demonstrated that these image-derived phenotypes provide complementary prognostic information independent of demographics and cardiovascular risk factors.

Conclusion: The study highlights the distinct genetic architecture and prognostic significance of cLD and cIMT, suggesting their potential for improving cardiovascular risk stratification in clinical practice.

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P18.064.C Genomic associations and mediation of serum 18-Oxocortisol levels at the KCNJ5 locus

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Background: Steroid hormones generated by the adrenal cortex form an extensive and tightly regulated causal cascade. In general population studies, genomic investigations are challenged by low steroid concentration causing data censoring and asymmetry, and by joint regulatory mechanisms which may prevent assignment of identified loci to specific steroids. To establish a modelling framework facing these issues, we piloted an association study between 18-Oxocortisol, a hallmark of KCNJ5-related aldosterone-producing adenomas, and the KCNJ5 locus itself.

Material and Methods: We quantified 5 mineralocorticoids, 7 glucocorticoids and 1 androgen, representing the zona glomerulosa, fasciculata and reticularis in 3204 individuals from the Cooperative Health Research in South Tyrol (CHRIS) study through serum LC-MS/MS. We fit a Tobit regression on 18-Oxocortisol controlling for age, sex, age-sex interaction, sex hormone intake, corticosteroid drugs and anti-hypertensive drugs. Inverse-normally transformed residuals were tested for association with 15,027 HRC-imputed variants in KCNJ5±1 Mb ($\alpha = 7.4 \times 10^{-5}$). Sensitivity analysis was performed by additionally adjusting for: each steroid in turn; all glucocorticoids; all steroids.

Results: 18-Oxocortisol was associated with two independent variants. Top association at rs949101 ($p = 1.5 \times 10^{-7}$) was attenuated by all-steroid adjustment ($p = 3.9 \times 10^{-6}$) but not by all-glucocorticoid adjustment ($p = 4.8 \times 10^{-8}$). Adjustment for single steroids identified a ~30% association attenuation and loss of significance when adjusting for the mineralocorticoid 11-Deoxycorticosterone ($p = 1.75 \times 10^{-4}$).

Conclusion: While results might suggest interplay between mineralo- and glucocorticoid metabolism, the apparent mediation likely reflects progesterone metabolism, which regulates the balance between 11-Deoxycorticosterone and the whole glucocorticoid chain levels. Caution is recommended when blindly applying mediation models to unknown pathways.

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P18.065.D Unraveling shared biological mechanisms of depression and Body Mass Index

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Background: Major Depressive disorder (MDD) and increased body mass index (BMI) are complex, highly polygenic, and interlinked phenotypes with a genetic correlation of 0.17. This study aims to elucidate the shared genetics and biological mechanisms of depression and increased BMI.

Methods and Results: We assessed whether BMI and psychiatric disorders share an underlying latent genetic factor using GenomicSEM. BMI, MDD, attention-deficit/hyperactivity disorder, and autism demonstrated positive loadings on the shared latent factor. Bivariate mixture modelling implemented in MiXeR revealed 9300 shared variants between BMI and MDD, 57% with concordant directions of effect. The to-date largest cross-disorder GWAS of MDD and BMI was performed, revealing 907 genome-wide significant loci with concordant directions of effect and a high posterior probability (> 0.9) of a contribution to the signal from both phenotypes. MAGMA property analysis identified a significant association of BMI-MDD risk genes with genes highly expressed in brain tissues. For concordant genome-wide significant loci sets of credible variants were integrated with functional genomics data in FLAMES to identify risk genes. This identified 582 potential shared risk genes, and analyses in Syngo, revealed an enrichment of these genes in postsynaptic regions. Risk genes were linked to cell types by estimating the single cell disease relevance score using single-cell RNA-seq data from brain derived cell types, which revealed that BMI-MDD genes are enriched in brain regions including cerebellar hemisphere, cerebellum etc.

Conclusion: There is substantial genetic overlap between MDD and BMI with shared cellular mechanisms in brain cells involved in each condition also individually.

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P18.066.A Rare variant effects on cell type-specific gene expression in immune cells from 4978 UK Biobank participants

Eva Holtkamp[1,2], *Marcel Mück*[3], *Magnus Wahlberg*[3], *Brian Clarke*[3], *Julien Gagneur*[1,2], *Nicole Soranzo*[4,5], *Oliver Stegle*[3,6]

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Background: Recent population-based single-cell eQTL studies have elucidated how common genetic variation influences expression across immune cell types. Still, the effect of rare variants on gene expression and their link to clinical traits remains poorly understood due to the lack of suitable datasets. Here, we leverage recently generated scRNA-seq profiles of PBMCs from 4978 UK Biobank (UKB) participants—the largest dataset to date integrating single-cell expression across 28 cell types with matched whole-genome-sequencing (WGS) and comprehensive medical records—to map the impact of rare variants on gene expression across immune cell types.

Material and Methods: For 4978 UKB participants with available RNA-seq, we processed matched WGS data to extract rare variants covering the full pre-mature transcript, including UTRs not covered by WES. We adapt our DeepRVAT1 framework to aggregate rare variant effects into transcript-level impairment scores, integrating diverse functional annotations such as missense, loss of function, splicing, RNA-protein binding, and chromatin effects.

Results: We will investigate cell type-specific associations between gene expression and immune-related clinical phenotypes, focusing on genes previously implicated in these phenotypes through rare variant association studies. Second, we use transcript-level impairment scores to i) study how rare variants affect transcript abundance in *cis*, and ii) to find cell-type specific regulatory interactions in *trans* by linking increased impairment to altered target gene expression.

Conclusion: Leveraging matched scRNA-seq and WGS data at an unprecedented scale, this study will elucidate the impact of rare variants on transcript stability and gene regulatory interactions across human immune cell types.

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P18.067.B Increased burden of rare variants in GWAS associated genes in familial multiple sclerosis

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Background: Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease affecting the central nervous system with many known genetic risk factors. Although genome-wide association studies (GWAS) have identified common genetic variants with small effects associated with MS, the role of rare variants with large effects in MS aetiology remains underexplored. We hypothesized that rare variants in MS-associated genes from GWAS studies (GWAS-associated genes) are more likely to contribute to familial MS (FMS) risk than to sporadic MS (SMS). Therefore, we aimed to assess the burden of rare, predicted pathogenic (RPP) variants in GWAS-associated genes in FMS and SMS patients compared to controls.

Material and Methods: Using a generalized linear model, rare genetic variants in 111 GWAS-associated genes were assessed in the whole-exome sequences of 87 FMS, 89 SMS and 3866 control cases.

Results: We demonstrate that RPP variants were significantly overrepresented in the FMS cohort whereas their frequency was not increased in the SMS cohort compared to controls (p-values 5.27×10^{-74} and 1.00, respectively). Six genes (*ALPK2, ANKRD55, INTS8, IQCB1, JADE2*, and *MALT1*) significantly contributed to the burden of RPP in the FMS group.

Conclusion: We conclude that rare variants in genes identified by GWAS might contribute to the genetic predisposition of familial MS patients.

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P18.068.C Analysis of *TMEM109* rare genetic variation underlying cardiovascular diseases and traits among 500000 individuals in the UK Biobank

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Background: Calcium ion (Ca^{2+}) leakage from the sarcoplasmic reticulum is a known contributor to heart failure in cardiac muscle. Our recent study identified Mitsugumin 23 (MG23) as an intracellular Ca^{2+}-conducting ion channel that facilitates this leakage in cardiac cells[1]. Our functional data demonstrated that knocking out of MG23 in a pressure-overload mouse model protects against left ventricular hypertrophy and cardiac dysfunction, linking it to cardiovascular disease (CVD) progression[2]. This current study utilizes UK Biobank[3] data to investigate the impact of rare variants in *TMEM109*, which encodes MG23, on CVD related phenotypes, and to pinpoint candidate variants for further functional verification.

Material and Methods: We analyzed whole-exome sequencing and phenotypic data from nearly 500,000 UK Biobank participants. Rare damaging *TMEM109* variants (minor allele frequency < 5%) were assessed for associations with 25 curated CVD phenotypes[4], including binary traits (hypertension, heart failure) and continuous traits (systolic and diastolic blood pressure).

Results: Our analysis identified a statistically significant gene-based association with decreased diastolic blood pressure (DBP) ($p < 2E-03$), driven by a missense variant ($p < 5E-03$), after multiple-testing adjustments. The *TMEM109* heterozygous profile revealed two deleterious missense variants carried by two individuals with CVD and these variants are linked to multiple CVD phenotypes, as supported by our analysis and data from Genebass, a public association study database[5].

Conclusion: Rare missense variants in *TMEM109* are significantly associated with decreased DBP, suggesting their protective role in CVD progression. Together with our experimental observations, we suggest that MG23 is involved in regulation of cardiovascular function.

C.O.I. Disclosure:

X. Guo: None. **S. Paracchini:** None. **S. Pitt:** None.

P18.069.D Metabolic pathways associated with alternative lengthening of telomeres and their therapeutic potential

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Background: Telomeres are nucleoprotein complexes that protect the ends of eukaryotic chromosomes from degradation, recombination, and end to end fusion. In cancer, most tumors activate various telomere maintenance mechanisms that enable them to elongate their telomeres and achieve cellular immortality. Alternative Lengthening of Telomeres (ALT) is a telomerase-independent mechanism employed by several aggressive cancers to maintain telomere length, contributing to their malignancy and resistance to conventional therapies.

Material and Methods: ALT-related metabolites were identified by analyzing interactions between 120 ALT-associated proteins and 106 Pan-Cancer oncogenes. Data were obtained from the Human Metabolome Database and interactions were extracted using the xml2 package in RStudio. Enrichment analysis was performed on the Small Molecule Pathway Database (SMPDB), KEGG PATHWAY, and MetaboAnalyst 5.0 to identify metabolic and drug pathways. A joint pathway analysis integrated ALT-related genes and metabolites. Finally, Cytoscape v3.9.1 was used to construct a high-confidence protein-metabolite interaction network.

Results: 39 ALT-related proteins were found to interact with 42 different metabolites in our analysis. Additional networking analysis revealed a complex interaction between metabolites and ALT-related proteins, suggesting that Pan-Cancer oncogenes may have an impact on these pathways. Three metabolic pathways have been primarily related with the ALT mechanism: purine metabolism, cysteine and methionine metabolism, and nicotinate and nicotinamide metabolism. We prioritized FDA-approved drugs (azathioprine, thioguanine, and mercaptopurine) that could target ALT-positive tumors through purine metabolism.

Conclusion: This work provides a wide perspective of the metabolomic pathways associated with ALT and reveals potential therapeutic targets that require further experimental validation.

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P18.070.A A Comparative Study of the Human Skin Microbiome in Korean and American Populations Using Public Metagenomic Data

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Background: Ethnicity significantly influences the human skin microbiome, affecting immune responses and skin health. While environmental and lifestyle factors contribute to microbiome variation, genetic background also plays a key role. This study aims to analyze the skin microbiome differences between Korean and American populations using publicly available metagenomic data.

Material and Methods: We utilized publicly available metagenomic datasets from SRAdb, selecting studies on Korean and American populations. Metadata was retrieved using EDirect, and raw data was processed through SRA Toolkit for FASTQ conversion. QIIME2 (2023.09) was employed for quality control, denoising, taxonomic classification (Silva 138). Alpha diversity metrics were compared using the Wilcoxon test. Beta diversity was assessed using PERMANOVA to evaluate microbial community differences and determine statistical significance. Taxonomic composition at the phylum and genus levels was analyzed to identify ethnicity-associated microbial patterns.

Results: Korean samples exhibited significantly higher alpha diversity than American samples (Chao1, OTU, Faith PD, $p = 2.00e-16$; Shannon, $p = 9.10e-04$; Simpson, $p = 1.60e-02$). Beta diversity analysis (PERMANOVA, $p = 5.00e-02$) showed distinct clustering. At the phylum level, Koreans had lower *Actinobacteriota* ($p = 4.20e-05$) and *Firmicutes* ($p = 4.39e-30$) but higher *Proteobacteria* ($p = 2.51e-04$). At the genus level, *Cutibacterium* was more abundant in Koreans ($p = 2.24e-04$), while *Corynebacterium* ($p = 9.07e-36$), *Staphylococcus* ($p = 5.79e-08$), and *Streptococcus* ($p = 9.26e-20$) were lower.

Conclusion: These findings highlight the impact of ethnicity on skin microbiome composition and emphasize the need for diverse representation in microbiome research to improve dermatological and precision medicine applications.

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P18.071.B Novel genetic variant regulating anti-spike IgG response to Covid-19 BNT162b2 vaccination

Alessandro Testori[1], *Antonella Mulas*[2], *Mara Marongiu*[2], *Monia Lobina*[2], *Mariagrazia Piras*[2], *SerGenCov Group*[3], *Alessandra Testa*[4], *Marcella Devoto*[5], *Anna Maristella Steri*[1], *Edoardo Fiorillo*[2]

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Background: The heterogeneity of the immune response to COVID-19 vaccine is partially explained by host genetic factors. Studies have demonstrated that inter-individual differences in the HLA region may influence the production of antibodies following vaccination. Their level is the result of humoral and cellular immunity regulating the response to infection and protecting against severe outcomes.

Material and Methods: To elucidate the mechanisms underlying antibody response to Pfizer-BioNTech vaccine, we conducted a GWAS testing more than 8 million variants on anti-spike immunoglobulin G levels in an Italian cohort of 1,968 fully vaccinated subjects. Association was adjusted for sex, age, BMI, smoking habit, and time elapsed between vaccine administration and blood draw. Flow cytometry data[1] were used to perform colocalization analysis.

Results: We identified a novel signal in the *CMTM8* gene and confirmed major signals at the HLA locus near the *HLA-B* gene, at the significance threshold of 5×10^{-8}. The novel variant affects Anti-S IgG levels after vaccination and is an eQTL for the *CMTM8* gene in blood and in immune cells ($CD4^+$ T cells, $CD8^+$ T cells, macrophages, and monocytes). Colocalization at the *CMTM8* locus suggests a shared genetic mechanism between antibody production and the expression of the CD66b marker in myeloid-derived suppressor cells.

Conclusion: These findings confirm the role of genetic regulation in modulating response to Pfizer-BioNTech vaccine. Genetic studies in diverse cohorts are important to define the involvement of genetic loci, including those outside the HLA region, in controlling antibody production following vaccination.

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P18.072.C Rett syndrome clinical variability: integrating MECP2 variant type, PRS and rare SNV/SV in additional genes

Heyran Huseynli[1,2], *Kristina Zguro*[1,2], *Giulia Brunelli*[2], *Simone Pesaresi*[2], *Elena Pasquinelli*[1,2], *Caterina Lo Rizzo*[1,2,3], *Roberto Canitano*[4], *Sabrina Buoni*[4], *Simone Furini*[5], *Romina D'aurizio*[6], *Susanna Croci*[1,2], *Margherita Baldassarri*[1,2,3], *Chiara Fallerini*[1,2], *Alessandra Renieri*[1,2,3]

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Background: Rett syndrome (RTT) is a neurodevelopmental disorder and a leading genetic cause of intellectual disability in females. While mutations in MECP2 are the primary cause, the huge clinical variability suggests additional genetic factors may contribute.

Material and Methods: A cohort of 245 RTT patients with known MECP2 mutations and balanced X inactivation in blood. Clinical severity assessed using the Clinical Severity Score (CSS). Polygenic Risk Scores (PRS) analyses performed for intelligence and epilepsy. Whole Exome Sequencing (WES) by NovaSeq6000 used to identify rare pathogenic variants other than MECP2. Long read- sequencing by Oxford Nanopore ongoing for adding clinical variability derived from structural variants.

Results: PRS analyses showed no significant contributions to epilepsy or cognitive severity alone. However, when integrated with MECP2 mutation type significant association with IQ < 40 become evident: early truncating versus missense (p. 0.0334) and early versus late truncating MECP2 variants (p.0.0063). Rare pathogenic/likely pathogenic variants were identified in 26% of patients, implicating genes associated with neurodegeneration, epilepsy, and scoliosis and work is ongoing for statistical integration.

Conclusion: The integration of PRS, MECP2 type of variants and rare SNV/SV in additional genes may contribute to new insights into RTT's genetic complexity and variability, advancing our understanding of the disorder.

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P18.073.D Trans-Ancestry Genome-Wide Association Study Meta-Analysis Identifies CTNNA2 as a Novel Risk Locus for Recurrent Miscarriage

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Background: Recurrent Pregnancy Loss (RPL) affects ~5% of reproductive-age women and is defined as the loss of two or more pregnancies before 24 weeks of gestation. Approximately 50% of RPL cases remain idiopathic. The genetics of RPL remains poorly understood, given difficulties with RPL phenotype definition. We investigated genetic susceptibility of RPL using a trans-ancestry genome-wide association study (GWAS) meta-analysis.

Material and Methods: We conducted ancestry-specific GWAS of self-reported recurrent miscarriage in 15,337/136,258 individuals among cases with 2+miscarriages and controls with no miscarriages of European (discovery), African, White, South/North Asian, East Asian descent using BOLT-LMM on the UK Biobank (UKBB). We also used TOPMed-imputed LUCAR (Lviv Ukrainian Cohort for Advancing Reproductive Health) GWAS from Western Ukraine. LUCAR includes 350 women with clinically confirmed idiopathic RPL and 454 control women with at least one healthy child. The GWAS of six datasets (31,024 cases/272,974controls) were combined using trans-ancestry meta-regression within MR-MEGA software tool.

Results: We identified a genome-wide significant association at rs4852592-G (OR(95%CI) = 1.0018(0.976–1.029), *P-value* $= 3.52 \times 10^{-8}$) located near *CTNNA2* gene. The association signal is primarily driven by the largest European-ancestry subset of UKBB. However, we validated ($P < 0.05$) this association at

rs4852592 in the East Asian UKBB (*P-value* = 0.0042) and LUCAR (*P-value* = 0.031) studies.

Conclusion: Our study reveals novel RPL risk locus near CTNNA2 gene, which acts as a link between the cadherin adhesion receptors and the cytoskeleton in regulating cell-cell adhesion, foetal neurodevelopment. It is elevated in the endometrium during implantation, potentially increasing RPL risk, and is nominally associated with gestational age, making it putative biological target.

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P18.074.A Whole-Genome sequence analysis of 600,000 individuals across diverse populations identifies rare alleles with large effects on type 2 diabetes

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Background: Genome-wide association studies of common phenotypes have primarily identified common variants with small effects.

Material and Methods: To uncover rarer variants with larger effects on type 2 diabetes, we analyzed 84 million variants, observed in at least 20 of 599,676 individuals with whole-genome sequencing data from the All of Us and UK Biobank studies. In each study, we used two definitions of type 2 diabetes, strict (n cases = 44,331), and broad (n cases = 73,598). The selection was based on available ICD9/10, SNOMED codes and Hba1c reading for AOU, and ICD9/10, READ2 and CTV3 codes for UKB, and self-reports for both. We performed pan-ancestry analyses using an additive model in REGENIE, adjusting for sex, age, age square, 40 PCs in UK Biobank and 16 PCs in All of Us.

Results: From the broad case definition, we identified 26 variants at Bonferroni significance ($p < 5.95 \times 10\text{-}10$) with a MAF below 0.05 spanning 8 distance independent loci. A variant at position chr20:44413714 had the smallest MAF and was present in less than 1 in 900 people. The odd ratios of the 26 variants ranged from 1.4 to 4.5. Thirteen variants were new and were all within 100 kb from known variants, and all in or near genes involved in the disease onset or progression (HNF-4, LINC02932, TCF7L2, TCF7L3).

Conclusion: Further studies are needed to assess independence of the variants from known signals, but our findings demonstrate the potential for detecting rare penetrant alleles both novel and previously known.

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P18.075.B Genome-wide meta-analysis conducted in three large biobanks expands the genetic landscape of lumbar disc herniations

Ville Salo[1], *Juhani Maatta*[1], *Eeva Sliz*[1], *FinnGen*[2], *Ene Reimann*[3], *Reedik Mägi*[3], *Estonian Biobank research team*[2], *Kadri Reis*[3], *Abdelrahman G. Elhanas*[3], *Anu Reigo*[3], *Priit Palta*[3], *Esko Tonu*[3], *Jaro Karppinen*[1], *Johannes Kettunen*[1]

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Background: Given that lumbar disc herniation (LDH) is a prevalent spinal condition that causes significant individual suffering and societal costs[1], the genetic basis of LDH has received relatively little research. Our aim was to increase understanding of the genetic factors influencing LDH.

Material and Methods: We performed a genome-wide association analysis (GWAS) of LDH (ICD-10:M51) in the FinnGen project and in Estonian and UK biobanks, followed by a genome-wide meta-analysis to combine the results. The meta-analysis comprised a total of 80 724 cases, and 748 975 controls. Sensitivity analyses were also conducted with more strict case definitions, by limiting LDH cases to the ones with radiculopathy (M51.1) and to those who have undergone surgery.

Results: In the meta-analysis, we identified 41 novel and replicated 23 known loci located more than 1 MB apart, each containing at least one variant associated with LDH at the genome level[2,3]. In sensitivity analysis, we also identified five novel loci associated with LDH-related surgical operations and replicated three previously associated loci.

Conclusion: The previously unreported LDH risk loci that we identified expand the understanding of the hereditary causes of LDH. While changes in disc-related structures and inflammation-related factors play a major role in the etiology of LDH, our results from multiple downstream analyses suggest that nervous system-related mechanisms may also be implicated. Overall, our research contributes to a deeper understanding of the genetic factors behind LDH, potentially paving the way for the development of new therapeutics and prevention methods for symptomatic LDH in the future.

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P18.076.C Characterization of `eoPRED` a placental DNA methylation predictor of early-onset preeclampsia and maternal vascular malperfusion

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Background: Abnormal placental pathology, particularly maternal vascular malperfusion (MVM), is associated with preeclampsia, fetal growth restriction, and adverse heart and brain development. The eoPRED tool is a DNAme-based method that estimates the similarity of a placental methylome to one of early-onset preeclampsia (EOPE, diagnosis<34 weeks). However, eoPRED has only been validated in several small datasets lacking pathology information. We hypothesized that eoPRED could quantitively assess placental pathology, therefore it could help identify factors contributing to placental dysfunction.

Material and Methods: We utilized Illumina EPICv1 DNAme data from placentas from the Stress Pregnancy and Health dataset (n = 493) with detailed pathology information (29% MVM), to assess associations between eoPRED score, pathology, and birth outcomes.

Results: The eoPRED score was higher in placentas from pregnancies affected by EOPE (n = 7) than LOPE (n = 42) p = 0.03) and no preeclampsia controls (n = 402, p < 0.01). In controls, eoPRED was negatively associated with gestational age (R = −0.18, p < 0.001), birth weight (R = −0.11, p = 0.03), and

placental efficiency ($R = -0.11$, $p = 0.03$). Furthermore, eoPRED correlated with the grade of MVM ($R = 0.34$, $p < 0.01$), but not other pathology findings. We identified 108 CpGs that were differentially methylated (FDR < 0.01, $|\Delta\beta|>0.05$) in association with high-grade MVM, including 8 CpGs in *TTTY14* on the Y chromosome.

Conclusion: eoPRED is correlated with EOPE, but also MVM pathology. Furthermore, eoPRED is associated with adverse pregnancy outcomes even in the absence of preeclampsia. This tool has utility for quantifying the pathophysiology of EOPE and other conditions associated with placental dysfunction and can be useful to assess in placental datasets where pathology information is unavailable.

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P18.079.B Genetic regulation of protein expression in prediabetes and type 2 diabetes

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Background: Prediabetes and type 2 diabetes (T2D) are characterized by insufficient insulin secretion and poor sensitivity, leading to complications and increased mortality. Genetic predisposition is known to play a role in these metabolic disorders. Not all individuals with prediabetes develop T2D, highlighting the importance of understanding the molecular mechanisms involved in this progression.

Material: To investigate these differences, we utilized genomics and plasma proteomics data from 450 individuals enrolled in the prediabetes lifestyle intervention study.

Results: Through differential protein expression analysis, we identified 225 out of 2523 proteins to be differentially expressed in 318 individuals with prediabetes compared to 88 individuals with T2D. Using a mixture model framework, we identified 65 shared protein quantitative trait loci (pQTL) effects suggesting overlapping genetic regulation of proteins. Further, we identified four differential pQTLs i.e., genetic variants with significant effect in only one condition, near genes coding for proteins SELE, ACP5, SCLY, and GYS1, showing significant positive effects on protein levels in prediabetes but not in T2D. These proteins were also found to be differentially expressed in prediabetes compared to T2D. Notably, knockout studies of SCLY in mice link it to glucose and lipid homeostasis, implicating pathways associated with fatty liver disease and glucose intolerance. Additionally, we identified 20 shared pQTLs with opposite effects between prediabetes and T2D, underscoring differences in genetic regulation between the two states.

Conclusion: Our study provides new insights into the molecular mechanisms underlying progression from prediabetes to T2D and a better understanding of the role of their genetic regulation.

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P18.080.C Genome-wide association study of cardiac conduction disease subtypes

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Background: Cardiac conduction disorders (CDs) represent interrupted impulse conduction at the SA-node (sick sinus syndrome, SSS), AV-node/His-bundle (atrioventricular block, AVB), or ventricular branches (left/right bundle branch block, LBBB/RBBB). With a lifetime prevalence of ~20%, they frequently require pacemaker implantation and may signify more widespread myocardial fibrosis. There is limited knowledge on the pathophysiology of CD subphenotypes.

Methods: We meta-analyzed genome-wide association studies (GWASs) of SSS (21,055 cases), AVB (80,052 cases), LBBB (42,181 cases) and RBBB (34,305 cases) across seven datasets of European ancestry. Shared associations with other cardiac phenotypes were explored.

Results: We identified 163 associations (94 novel associations at 84 loci) across the four GWASs including AVB missense variants in the energy metabolism genes GFM1 and GMPR and the myopathy-linked MLIP and SUN1 genes. Variants at RNF207, BAG3, NKX2-5 and CCDC141 preferentially associated with high-degree AVB. Rare missense variants in A2ML1 and ion channel gene TRPM4 associated with LBBB and RBBB, respectively. RBBB variants were enriched in regulatory regions of GATA4/ESRRG pathways, known to be involved in cardiac hypertrophic response. We did not observe causal linear relationships of cardiomyopathies with CDs but there were multiple shared associations, including a previously unreported CD variant association with hypertrophic cardiomyopathy at CCDC141.

Conclusion: There is limited overlap between genetic backgrounds of different types of CDs. CDs have complex allele-specific relationships with cardiac remodeling phenotypes and GATA4/ESRRG-mediated hypertrophic response likely plays a role. The findings may aid in future therapeutic developments and individual risk assessment, e.g. for CD severity and cardiomyopathy.

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P18.081.D Large-scale proteomics of CSF and plasma identifies biomarkers and provides insights into Parkinson's disease pathogenesis

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder with a long preclinical phase, where neuronal degeneration begins before motor symptoms appear. Its complexity has hindered biomarker discovery and therapy development. High-throughput proteomics enables fluid biomarker research, with cerebrospinal fluid (CSF) offering central nervous system (CNS)-specific insights. However, standardized CSF collection remains a challenge. DDC has emerged as a potential diagnostic biomarker, though drug effects remain uncertain. Additionally, integrating genomics with proteomics facilitates the identification of key players in PD pathogenesis.

Material and Methods: We measured protein levels in CSF from PD patients and neurologically healthy controls, as well as in plasma from population-based cohorts. Our analyses included a CSF pQTL analysis, meta-analyses of CSF and plasma proteomics comparing early-phase PD patients to controls, and longitudinal protein correlation analyses, using CSF and plasma samples collected over eight years to assess associations between protein levels, PD progression scores, and disease duration.

Results: We present evidence supporting HIP1R as a causal PD gene, likely contributing to pathogenesis by disrupted synaptic vesicle turnover revealed by decreased CSF protein levels. Furthermore, we confirm DDC as a potential diagnostic biomarker in CSF, with elevated levels in CSF, but not plasma, of drug-naïve patients. Finally, we identify integrins, including ITGAV, ITGAM, ITGA11, and ITGB2, as promising plasma biomarkers for neurodegeneration detection and progression.

Conclusion: Our large-scale approach reveals clinically relevant protein signatures, underscoring distinct PD diseases mechanisms in the CNS versus systemic compartments.

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P18.082.A Longitudinal analysis of genetic and environmental interplay in human metabolic profiles and the implication for metabolic health

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Background: Metabolites' levels are regulated by a complex interplay of genetic predispositions and environmental exposures. Understanding how genetics and environmental factors shape metabolic profiles is crucial for advancing metabolic health.

Material and Methods: Longitudinal data of 527 metabolites, measured during a 2-year wellness study involving 101 healthy participants, were integrated with paired whole-genome sequencing, proteomics, extensive clinical phenotyping and lifestyle questionnaires. GWAS and multivariate analyses were performed to evaluate the regulation of the human metabolome. Prediction models based on central nodes in the protein-metabolite network were trained to predict multiple metabolic diseases.

Results: Our findings highlighted a considerable role of genetics in regulating the human metabolome, identifying 22 plasma metabolites as genetically predetermined. Environmental factors such as seasonal variation, weight management, smoking, and stress also significantly influenced metabolite levels. We developed an integrative metabolite-protein network comprising 5,649 significant protein-metabolite pairs and identified 87 causal metabolite-protein associations under genetic regulation, validated in an independent cohort. Key proteins representing individual metabolic profiles were identified and validated in the UK Biobank, showing great potential for predicting metabolic diseases and metabolic risk assessment.

Conclusion: In this study, we provided a comprehensive annotation of the genetic, clinical, proteomic and environmental factors that regulate the levels of 527 metabolites in plasma. We showed that each individual possesses a unique and stable protein-metabolic profile, determined primarily by genetics, sex and body composition, emphasizing metabolic individuality. Moreover, our results demonstrated the importance of plasma

proteins in capturing individualized metabolic variabilities and monitoring metabolic health.

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P18.083.B Integrating genetics and metabolomics: A genome-wide association study of plasma metabolite profiles in people living with HIV

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Background: People living with HIV (PLWH) exhibit premature aging and a higher prevalence of age-related conditions such as cardiovascular and chronic kidney disease, despite effective antiretroviral therapy (ART). The underlying biological mechanisms contributing to this phenomenon remain poorly understood. Metabolomics, the analysis of small molecules in biological samples, offers a powerful tool for investigating the molecular mechanisms of health perturbations. Previous studies indicate that ART only partially addresses the metabolic disturbances caused by HIV and might contribute to additional metabolic dysregulation. We here integrate metabolomic and genomic profiling to explore the biological underpinning of age-related comorbidities in PLWH.

Material and Methods: Using a genome-wide association study framework and untargeted metabolome measurements, we searched for assocations between human genetic variants and the plasma concentrations of 1930 metabolites among 1244 individuals from the Swiss HIV Cohort Study.

Results: We identified 27 significantly associated metabolites across 12 genetic loci, including metabolic enzyme genes such as NAT8 and FUT2. Expression quantitative trait loci analysis confirmed plausible biological connections between genetic variants and metabolites. Of the 27 associations with metabolites, 8 had been reported in previous studies. We identified genetic links to plant-derived metabolites, uncovering novel genetic regulation of exogenous compound metabolism in humans. Finally, Mendelian Randomization was applied to investigate the causal relationship between these metabolites and age-related diseases.

Conclusion: We conducted a genome-wide search for associations with plasma metabolites in PLWH, revealing some genetic-metabolic links that were not detected in the general population and could be linked to age-related comorbidities in PLWH.

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P18.084.C Understanding the genetic basis for sleep and psychiatric disorders

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Background: Sleep is vital for mental and physical health, but modern lifestyles often lead to poor sleep. Sleep and psychiatric disorders have a bidirectional relationship; sleep problems can worsen mental health, and psychiatric conditions can disrupt sleep. While it is known that both sleep and psychiatric disorders have a strong genetic basis, the overlap of their genetic risk factors remains unknown.

Material and Methods: In this study, we leveraged genome-wide association study (GWAS) data of over 50 sleep and psychiatric phenotypes and performed genetic correlation analysis to understand the shared genetic basis underlying these conditions. Further, we explored the enrichment of SNP-based heritability of these disorders in various tissues by stratified linkage disequilibrium score regression (LDSC) analysis using publicly available gene expression and chromatin data from a wide range of human, mouse, and rat tissues.

Results: We found significant negative correlations between sleep duration and post-traumatic stress disorder ($R = -0.227$, $p = 4.12e\text{-}4$), and between chronotype and autism spectrum disorder ($R = -0.172$, $p = 2.41e\text{-}7$). Additionally, we observed positive genetic correlations between nightmares and mood instability ($R = 0.6$, $p = 4.73e\text{-}6$), and between insomnia and depression ($R = 0.457$, $p = 6.72e\text{-}91$). LDSC analysis showed the enriched heritability of most sleep and psychiatric disorders in tissues of the central nervous system, particularly brain regions including the prefrontal cortex, angular and anterior gyrus ($p < 0.05$).

Conclusion: Overall, our findings highlight the shared genetic basis of sleep and psychiatric disorders, and highlight tissues of the central nervous system as most relevant for understanding disease heritability.

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P18.085.D Postnatal depression GWAS in the Genetic Links to Anxiety and Depression (GLAD) Study and UK Biobank

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Background: Postnatal depression (PND) is a common subtype of major depression disorder (MDD) that is often under-researched in psychiatric genetics. In this study we present results of a genome-wide association study (GWAS) of PND.

Material and Methods: We defined PND in the Genetics Links to Anxiety and Depression (GLAD) Study and the UK Biobank using a combination of self-report and diagnostic algorithms. The combined sample size in the two cohorts included 6,413 cases and 18,122 controls ($N = 24{,}535$). We performed a GWAS in REGENIE

and estimated SNP heritability using the LDSC tool. Functional mapping and annotation were done using FUMA.

Results: Our GWAS results identified 1 genome-wide significant signal on chromosome 13, rs12224658, near the *OR4C4/OR4A5* genes. This signal has not been reported in current large scale GWAS of PND or MDD. The observed SNP heritability estimate was 5.8%. Ongoing work focuses on refining the definition of PND in GLAD and UK Biobank to increase sample sizes, followed by meta-analysis with the latest large-scale study of PND. Genetic correlation with other traits will be estimated in the combined dataset.

Conclusion: We performed a GWAS of PND in the UK utilising data from the largest study of depression in the world and UK Biobank and identified one genome-wide significant signal. Results from our work will build on existing research and improve our understanding of the distinct genetic aetiology of PND compared to MDD.

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P18.087.B First genome-wide association study of chronic lung allograft dysfunction

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Background: Lung graft survival remains insufficient, with only 50.6% survival at 5 years post-transplantation. Chronic lung allograft dysfunction (CLAD) is the primary barrier to long-term survival. Understanding the molecular mechanisms underlying CLAD is critical for improving outcomes. We performed the first genome-wide association study (GWAS) investigating donor and recipient's genetic factors associated with CLAD.

Material and Methods: CLAD is defined by a ≥20% decline in forced expiratory volume from baseline (3 months post-transplant without infection or other causes). We genotyped 392 European donor-recipient pairs from the multicentric COLT cohort (Cohort in Lung Transplantation) using the Affymetrix Axiom PRMA microarray. We tested 7 million SNPs for association with CLAD using multivariate logistic regression models corrected for age, sex, initial disease and genetic ancestry. The GWAS was performed at three levels: donors-only, recipients-only, and donor-recipient mismatches.

Results: No significant associations were found in donor genotypes or donor-recipient mismatches. However, 13 recipients SNPs were significantly associated with CLAD ($P < 5 \times 10^{-8}$). Our top signals (34% in CLAD vs 66% in non CLAD recipients, $P = 2.18 \times 10^{-9}$, $OR = 0.39$) encompassed the *PLXDC2* gene, previously associated with the risk for bronchopulmonary dysplasia. Importantly, we demonstrated with scRNAseq data from lung explants ($n = 4$) that *PLXDC2* was mostly expressed in monocytes and strongly down regulated in CLAD vs healthy controls ($P = 2.22 \times 10^{-16}$).

Conclusion: This first GWAS in lung transplantation displaying a biologically relevant candidate gene from the recipient's genome, suggesting its role in CLAD pathogenesis. Our study calls for larger genomic initiatives in lung transplantation to increase the power of signal discovery.

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P18.088.C Transcriptome-wide association study and network analysis reveal tissue-specificity of genetic determinants of multiple sclerosis severity

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Background: Multiple sclerosis (MS) is a major cause of neurological impairment in young adults. While effective therapies are available for the relapsing-remitting form, progression of MS has very limited treatment options. To improve understanding of the biological substrates of MS severity, we integrated genome-wide association studies (GWAS) of disease severity with gene expression data, performing tissue-specific transcriptome-wide association studies (TWAS), followed by a thorough investigation of involved network modules.

Material and Methods: We meta-analysed statistics from two GWAS on MS severity: the multi-centric study performed by the International Multiple Sclerosis Genetics Consortium ($N = 12,584$) and a study involving an Italian cohort ($N = 1,286$). To perform TWAS, we used gene-expression predictive models in 12 brain regions from GTEx v8, using FUSION tool to test association of genetically predicted expression with severity endpoint. The TWAS statistics were projected onto corresponding tissue-specific interactomes from HumanBase. Using "dmfind" network analysis

tool, we scored modules based on the presence of connected genes with significant TWAS statistics and tissue-specificity.

Results: Overall, TWAS was based on meta-analysed statistics for 7,891,356 imputed variants. We found genes whose genetically controlled expression was significantly associated ($p < 0.05$) with MS severity in each tissue (50–220 genes). Network analysis revealed a set of "high scoring" gene modules (10–80 genes), characterized by high tissue specificity and high TWAS statistics. Interestingly, spinal cord emerged as a tissue with top-scoring modules.

Conclusion: Our analysis provided evidence of tissue-selective manifestation of MS severity genetic determinants. Further work is required to validate and interpret the molecular mechanisms underlying such tissue-specificity.

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P18.089.D Bulk and single-cell transcriptomic insights into treatment-induced changes in tuberculosis patients from Tanzania

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Background: Transcriptomic analysis has allowed researchers to investigate how an individual's gene expression is modulated by *Mycobacterium tuberculosis* infection and antimycobacterial drugs [1-2]. However, most studies have only used bulk RNAseq, which cannot ascertain how cell-type composition and cell-type specific responses impact global expression patterns. Thus, we sought to characterise these potential influences using pre- and post-treatment clinical samples from Tanzanian patients.

Material and Methods: Blood samples were collected between 2019–2021 from 24 patients immediately before and six months after treatment. scRNAseq was performed by following BD Rhapsody's mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol. Bulk RNAseq was performed by following the Illumina Stranded mRNA Prep Protocol. Bioinformatic analysis was performed in R.

Results: Our bulk transcriptomic analysis confirmed previously published gene signatures, notably the downregulation of immunoglobulin-associated genes after treatment (e.g. IGHA1-2, IGLV2-8). Through our scRNAseq analysis, we showed that shifts in both cell-type composition and cell-type-specific expression changes impacted the bulk expression patterns. The proportion of B cells and CD4+ memory T cells increased following treatment ($p = 0.048$). Furthermore, natural killer cells and monocytes exhibited differential gene expression profiles distinct from the bulk expression pattern.

Conclusion: These findings highlight the value of scRNAseq in recontextualising gene expression patterns observed in bulk RNAseq, facilitating a greater understanding into the complex biology of tuberculosis. Further analysis into applying deconvolutional pipelines on publicly available bulk RNAseq datasets will help assess the generalisability of our results.

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P18.090.A Genetic Drivers of Alzheimer's Disease Pathology: A GWAS and QTL analysis in a novel European neuropathological cohort

Celeste Laureyssen[1,2], *Fahri Küçükali*[1,2], *Jasper Van Dongen*[1,3], *Klara Gawor*[4], *Alicja Ronisz*[5], *Markus Otto*[6], *Christine A.F. Von Arnim*[7], *Philip Vandamme*[8,9], *Rik Vandenberghe*[9,10], *Dietmar R. Thal*[4,11], *Kristel Sleegers*[1,2]

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Background: Alzheimer's disease (AD) is a heterogeneous disorder with a substantial genetic component. Traditional genome-wide association studies (GWAS) rely on clinical diagnoses of large cohorts, which are compromised by phenotypic heterogeneity. We conducted GWAS in a novel European neuropathological cohort ($n = 414$) with detailed neuropathological characterization to identify genetic associations with specific AD-related lesions.

Material and Methods: AVITI low-coverage whole-genome sequencing was performed at 1x coverage. SNPs and indels were imputed using GLIMPSE2, referencing the 1000 Genomes Project panel. Association testing was conducted in PLINK2. Variants reaching genome-wide significance ($p < 5 \times 10^{-8}$) and notable subthreshold loci were evaluated for potential roles as quantitative trait loci (QTLs).

Results: We identified genome-wide significant and suggestive loci contributing to the heterogeneity of AD risk. In addition to APOE, we discovered an intronic variant in GYPE and a variant upstream of ADAMTS16, both showed nominal associations with ABC score, reflecting AD pathology. Both were significantly linked to Braak staging (Tau neuropathological score), suggesting a potential role in tau-related lesions. Significant associations with co-morbidities were found, like granulovacuolar degeneration (necroptotic cell death), with an intronic variant in XAF1, and an intronic variant in NRF1 associated with Hirano body scores (actin aggregates).

Conclusion: Despite sample size constraints, we identified genome-wide significant associations with AD-related lesions by controlling for phenotypic heterogeneity. Results from QTL analyses highlight the molecular relevance of several loci and suggest potential novel risk genes. Moving forward, we will prioritize replication and meta-analysis in independent cohorts, functional validation and targeted resequencing.

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P18.091.B Gut microbiome composition and function reflect socioeconomic deprivation

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Background: Socioeconomic status (SES) correlates with adverse health outcomes, but underlying biological mechanisms remain unclear. We examined the influence of area-level deprivation, measured by the Townsend Deprivation Index, on gut microbiome composition and function (using shotgun metagenomics). We also explored how the gut microbiome mediates the effects of deprivation on metabolic and mental health outcomes.

Material and Methods: In 1,390 female individuals from TwinsUK, we investigated changes in microbial diversity, composition and function across deprivation levels using linear mixed models as implemented in Maaslin2 adjusting for age, BMI, family and multiple testing (FDR < 0.2). XGboost models assessed whether selected bacteria could distinguish between the most and least deprived groups. Mediation analysis examined the role of microbes in linking social deprivation to metabolic and mental health outcomes.

Results: Individuals with higher Townsend deprivation exhibited reduced alpha diversity (Jonckheere-Terpstra $P < 0.001$) and distinct shifts in microbial composition were observed across deprivation levels (PERMANOVA $P = 0.001$). Twenty microbial species were linked to deprivation and moderately distinguished deprived groups (AUC = 0.73). Altered energy metabolism pathways were identified in deprived individuals. Townsend deprivation was linked with higher levels of glucose, overweight and anxiety. Two social deprivation linked microbial species were consistently associated with these clinical outcomes, with *Intestinimonas_massiliensis* and *Ruminococcaceae_unclassified_SGB15236* mediating the relationship between deprivation, overweight and glucose levels. *Intestinimonas_massiliensis* also mediates the association between deprivation and anxiety.

Conclusion: Socioeconomic deprivation reshapes microbiome composition. Our data highlights the mediating effect of the gut microbiome on mental and metabolic health disparities.

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P18.092.C Mapping genetic blueprints of human plasma blood proteins: A GWAS study in KORA

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Background: Genome-wide association studies (GWAS) analyzed thousands of genetic variants to uncover statistical associations with diseases and traits. Integrating genomics with protein levels in human blood samples provides new understanding of key loci. Protein Quantitative Trait Loci (pQTLs) offer substantial effect sizes and complement other analyses, as protein levels are often directly implicated in disease processes. We integrated proteomic profiling across three timepoints in KORA (Cooperative Health Research in the Region of Augsburg, Germany) to investigate temporal trends between genetic variation and protein expression.

Methods: We explored a novel plasma proteomics longitudinal dataset acquired through mass spectrometry assay. We utilized samples measured in baseline KORA S4 = 3652, 7-years follow-up F4 = 2854, 14-years follow-up FF4 = 2005 participants. The SNP-protein associations (842 relative protein concentrations, post-QC) were tested using Matrix-eQTL by modeling the effect of genotype as additive linear model. Age, sex and BMI were included as covariates.

Results: We identified 4,133 significant genetic associations linked to 537 proteins. An interactive online platform with supplementary material is being developed for easy result access. We validated our findings by cross-referencing these associations with MS-based peptide data. Subsequently, we examined association between age and proteins while accounting for other covariates (SNP, time, sex, BMI) using a linear mixed model. 1,472 SNPs were identified as associated with age after Bonferroni correction. Ongoing analysis examines the SNP-age associations for novelty by cross-referencing with existing literature.

Conclusion: Our goal is to prioritize novel associations for deeper investigation, including their potential functional implications and mechanisms.

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M. Fuchs: A. Employment (full or part-time); Significant; Research Unit of Molecular Epidemiology, Helmholtz Zentrum Munich, Neuherberg, Germany, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany. **J. Adam:** A. Employment (full or part-time); Significant; Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany. **B. Linkohr:** A. Employment (full or part-time); Significant; Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany. **A. Peters:** A. Employment (full or part-time); Significant; Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany, Chair of Epidemiology, Faculty of Medicine, Ludwig-Maximilians-University München, Munich, Germany. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Center for Cardiovascular Disease (DZHK), Partner Site Munich Heart Alliance, Munich, Germany. **K. Suhre:** A. Employment (full or part-time); Significant; Weill Cornell Medicine Qatar, Doha. **C. Gieger:** A. Employment (full or part-time); Significant; Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; German Center for Diabetes Research (DZD).

P18.093.D Leveraging same-cell single-nucleus multiomics across PBMCs from 271 donors to understand how genetic variation affects gene regulation

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Background: Many, mostly non-coding, genetic variants have been linked to disease. Through single-cell eQTL analysis we have

linked such variants to (celltype-specific) gene expression changes. However, nominating both causal and relevant variants in a locus remains difficult.

Material and Methods: To better understand the upstream regulatory mechanisms through which genetic variants act, allowing better prioritization of likely causal variant disrupting regulation, we performed single-nucleus multiome (RNA + ATAC) experiments across 318 blood samples from 271 individuals.

Results: 5,365 genes showed an eQTL effect (15% of top effects are in open chromatin, and 69% are co-localizing with a caQTL in the matching context) across the six major blood cell types. For these co-localizing QTL effects, 92% were concordant across modalities. We then identified cis-regulatory elements where chromatin levels of genomic regions correlated with expression levels of specific genes, and found examples where this correlation was modulated by genetic variation and stimulation status. For example, the correlation between peak Chr1:89,556,742-89,557,773 and gene *LRRC8B* was modulated by a SNP located in the peak. Interestingly, this genotypic effect became weaker after pathogen stimulation, indicating that openness is the rate-limiting factor for *LRRC8B* transcription in the steady state, as opposed to the abundance of upstream regulators in the stimulated setting.

Conclusion: By taking advantage of same-cell open chromatin and expression information, we were able to better nominate and understand likely relevant variants and provide a first hint that the rate-limiting regulatory step of these variants may be context-dependent.

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P18.094.A Uncovering genetic variations in the KIV-2 variable number tandem repeat of the *LPA* gene: implications for lipoprotein(a) concentration and cardiovascular risk

Silvia Di Maio[1], *Peter Zöscher*[1], *Hansi Weissensteiner*[1], *Lukas Forer*[1], *Johanna F. Schachtl-Riess*[1], *Stephan Amstler*[1], *Gertraud Streiter*[1], *Cathrin Pfurtscheller*[1], *Anna Köttgen*[2], *Kai-Uwe Eckardt*[3,4], *Florian Kronenberg*[1], *Stefan Coassin*[1], *Sebastian Schönherr*[1]

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Background: Variable Number Tandem Repeats (VNTRs) are repetitive DNA sequences in the human genome. Over 100 VNTRs are located in protein-coding, medically relevant genes, like the KIV-2 VNTR in the *LPA* gene. *LPA* almost monogenically controls the concentration of the strong cardiovascular risk factor lipoprotein(a). Due to its structure, the KIV-2 VNTR analysis is often limited to resource-intensive approaches. Thus, VNTRs cannot be resolved in available biobank-scale sequencing data and are largely missed in GWAS.

Material and Methods: We previously published an in-silico workflow to detect intra-repeat variation at scale in exome data. Here, we applied this method for the first time to 4,867 whole-exome sequencing samples from the German Chronic Kidney Disease study and implemented a novel VNTR-wide association testing approach. Significant signals were further investigated with quantile regression on median Lp(a) concentrations and Cox regression on cardiovascular risk in 186,607 UK Biobank samples.

Results: We replicated all major findings from our previously established PCR-based approaches. Furthermore, we identified four novel significant associations with increasing or decreasing Lp(a) concentrations (Table 1), and even one new significant association with decreased CVD risk in UK Biobank (position 515A>G). Most importantly, the KIV-2 SNPs reduced the missing heritability of the Lp(a) variance, improving state-of-the-art polygenic risk scores ($r^2 = 44\%$) by about 7%.

Conclusion: Our framework unlocks variant calling and characterization in the KIV-2 VNTR from sequencing data at scale and can be used as a blueprint for other VNTRs and traits.

Novel associations between KIV-2 variants and Lp(a) concentration

Position within KIV-2 reference	Number of Carriers	Quantile regression beta [95% C.I.] mg/dL	p-value
672C>T	158	+11.88 [5.79;17.97]	3.39e-04
4968G>A	2,803	+3.52 [1.82;5.22]	1.98e-04
584C>T	179	−3.23 [−5.16;−1.3]	3.55e-06
515A>G	1,745	−9.65 [−11.67;−7.62]	1.88e-122

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P18.095.B Multi-trait meta-analyses and gene prioritization based on co-morbidity categories provide novel insights into the genetic architecture of hypothyroidism

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Background: Hypothyroidism is a common endocrine condition with extensive heterogeneity in symptoms, co-morbidities, and treatment effects. We studied the genetic underpinnings of

hypothyroidism in the context of its co-morbidities for an improved understanding of the disease biology and mechanisms.

Material and Methods: By leveraging publicly available genome-wide association study (GWAS) summary statistics, we aimed to (1) identify new genetic associations for hypothyroidism, and (2) explore the genetic overlap across 26 genetically correlated conditions, by performing seven co-morbidity-category-based multi-trait GWAS meta-analyses (MTAG), boosting statistical power by joint analysis of multiple traits. Based on the new associations identified, gene prioritization was carried out to detect and characterize the potentially functional genes using two parallel SNP-to-Gene methods; (1) Otargen, based on Open Targets Genetics, and (2) combined-S2G-Framework (cS2G).

Results: One-hundred-fourteen new variants in 92 loci for hypothyroidism ($p < 5 \times 10^{-8}$) lead to 1,134 genes prioritized. Twenty-six of 1,134 genes were prioritized as 'top-scoring' across the methods, including 16 nearest protein-coding genes such as *GLIS3, NRG1, KCTD5*, and *SLC25A37*, that had significant thyroid expression patterns. Among the genes of interest, *GLIS3* variants were previously associated with multiple co-morbidities of hypothyroidism, including rheumatoid arthritis, type 1 diabetes, depression, as well as thyroid-related traits, blood cholesterol, and sex hormone levels. *GLIS3* loss-of-function mutations lead to hyperglycemia, hyperinsulinemia, and congenital hypothyroidism.

Conclusion: MTAG revealed new genetic associations for hypothyroidism based on publicly available data, providing novel insights into its genetic architecture and advancing the understanding of the shared genetics and common biological processes between hypothyroidism and its co-morbidities.

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P18.096.C Identification of novel variants influencing the expression of drug-targetable genes involved with Multiple Sclerosis (MS) through a combination of insilico and invitro techniques

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Background: Multiple Sclerosis (MS) is a complex autoimmune disorder. Starting from 200 MS risk loci from Genome-Wide Association Studies, we focus on 5 regions showing replication in an Italian cohort. To overcome fine mapping limitations due to Linkage Disequilibrium we utilized Massively Parallel Reporter Assays (MPRA) to discover associated causative variants.

Material and Methods: We applied MPRA on regions encompassing SNPs in high LD (r2>0.77). We used mpralm for significance filter and to obtain the logFC of each SNP. We then used MotifBreakR and MEME suite to find Transcription Factors (TF) that bind to MS risk alleles. Open Target Genetics allowed the prediction of target genes and identification of drugs.

Results: We identified variants altering the expression of 4 different genes out of the 5 tested through preferential binding of TF. MEF2B binding on Rs1883832 and AHR binding to RS6974022 on the CD40 region targeting the CD40 gene with Iscalimab as a drug, TCF4, and E2F4 binding on 3 variants in the PRDX5 region with VEGF-B as a target gene, VEGF154 and CONBERCEPT as drugs. Lastly, TGIF2 and PROX1 binding on 2 variants in the IFNGR2 region with the IFNGR2 gene as a target, and INTERFERON GAMMA-1B as a drug, and TBX3 binding on 1 variant in the TEC region targeting the TEC gene with Capivarsetib as a drug. As a follow-up, the FREP assay will prove the binding of these TF to the sequences.

Conclusion: The combination of in silico and in vitro tools is able to identify variants primarily associated with complex diseases.

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P18.098.A Clinical implications of a structural variant in the iron transport gene SLC11A2: Insights from two large Danish cohorts

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Background: *SLC11A2* encodes divalent metal transporter-1, essential for iron absorption. A rare 3.55 kb deletion in the 3′-untranslated region of *SLC11A2* was linked to iron deficiency anemia in homozygote Icelandic carriers. Heterozygotes also showed reduced expression of iron response element positive *SLC11A2* transcripts. This study examines the impact of this structural variant on phenotypes in two large Danish cohorts.

Material and Methods: We analyzed 119,987 participants from the Danish Blood Donor Study (DBDS) and 378,977 from the Copenhagen Hospital Biobank (CHB). Genotyping was performed using the Illumina Global Screening Array. Carrier status was identified via GraphTyper2. Biomarker data, prescription use, and diagnoses were obtained from Danish health registries and DBDS data. Associations between carrier status and outcomes were evaluated using Cox, logistic, and linear regression models. Random effects models were used for repeated biomarker measurements.

Results: The minor allele frequency was 0.0072 across cohorts. No homozygotes were identified in DBDS and fewer than five in CHB. Heterozygote carriers had lower ferritin levels (DBDS: 8–9% lower; CHB: 7–9% lower). In DBDS women, heterozygote carriage was associated with higher risks of iron deficiency (HR 1.34, 95%CI 1.13–1.61), low hemoglobin (HR 1.36, 95%CI 1.21–1.53), and oral iron treatment (OR 1.82, 95%CI 1.21–2.61). Male carriers had 22% lower hepcidin levels (DBDS) and increased iron deficiency risk (CHB: HR 1.21, 95%CI 1.02–1.43) but normal hemoglobin levels. No associations were found with cardiovascular disease or diabetes.

Conclusion: This study highlights the significant impact of the *SLC11A2* structural variant on iron status in healthy and patient populations.

C.O.I. Disclosure:

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P18.099.B Colocalization of a COPD lung function GWAS and a single-cell lung eQTL study

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Background: Genetic association studies of chronic obstructive pulmonary disease (COPD) and its diagnostic, spirometry FEV1/FVC ratio, have identified hundreds of associations and their effector genes through bulk expression quantitative trait loci (eQTL) studies, such as the *DSP* (desmoplakin) gene, but the responsible cell types remain unknown. We sought to identify effector genes and cell types using single-cell lung eQTL data.

Material and Methods: We colocalized genome-wide significant variants from a lung function FEV1/FVC GWAS in > 580,000 multi-ancestry participants (Shrine 2023) with single-cell lung eQTL from 114 donors (Natri 2024). We included overlapping variants between GWAS and eQTL datasets with 100 kb flanks of the lead lung function variants using a prior probability of shared effect of 1e-5 (coloc R package). We tested across 38 cell types and considered colocalization at a posterior probability (PPH4) of 0.7.

Results: Testing 142 regions, we identified 12 loci with PPH4 > 0.7 in any cell type. Noteworthily, we found a PPH4 of 0.99 at the *DSP* locus (lead variant, rs2076295, in contrast to the variant previously reported to colocalize at this locus), in type 2 alveolar cells, and a PPH4 of 0.89 in alveolar type 1 cells.

Conclusion: In an eQTL colocalization analysis, we identified 12 COPD-related lung function loci that colocalized in single-cell eQTL studies, including *DSP*. rs2076295 likely plays a cell type-specific role in COPD susceptibility. Future work will include examination of the relationship of single-cell and bulk eQTL.

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P18.100.C GWAS and functional genomics reveal candidate susceptibility genes for endometrial cancer

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Background: Endometrial cancer is one of the most common malignancies worldwide, with ~420,300 new cases diagnosed in 2022. Only four drugs have received FDA approval for endometrial cancer in the last four decades. Genetic studies of disease offer a promising approach to identify new effective drug targets. Indeed, it has been observed that there is a four-fold increase in the proportion of drug targets that are supported by relevant genetic evidence between pre-clinical development and regulatory drug approval. Thus, we propose that susceptibility genes at endometrial cancer GWAS risk loci will provide novel therapeutic targets underlying disease causality.

Material and Methods: Through GWAS we have identified 21 genetic loci associated with endometrial cancer risk, resolved to 497 fine-mapped candidate causal risk variants. We performed various functional genomic assays to map regulatory features in endometrial models (ATAC-seq, H3K27Ac-HiChIP, H3K27Ac-CuT&TAG). These and publicly available datasets were integrated using six complementary approaches to prioritise functional endometrial cancer risk variants and their potential regulatory targets.

Results: Using LD score regression, we demonstrated significant enrichment of endometrial cancer heritability within regulatory elements mapped in endometrial models. We prioritised 73 candidate effector genes of endometrial cancer GWAS-risk variation, including three genes identified by all six approaches (*PPARG*, *BPTF* and *LINC02523*). Additionally, five endometrial cancer driver genes were found.

Conclusion: Regulatory elements in endometrial models likely mediate the effects of risk variation, revealing candidate effector genes. We prioritised *BPTF*, a promising therapeutic target implicated in several cancers, including endometrial cancer.

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P18.101.D A genetic atlas of human protein complexes in 1000 adults from the Orkney Isles

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Protein complexes are fundamental for biological processes and molecular interaction**s**, yet their roles in human health remain largely unexplored. Quantifying protein complexes at a population scale is of great challenge.In this study, we developed and integrated advanced experimental assays with AI-assisted computational methods to achieve **high-throughput scoring of over 3,000 human protein complexes** in more than 1,000 individuals from the isolated Orkney Islands.We provide a **comprehensive genetic atlas** of human protein complexes, identifying **2,846 protein complex QTL (pcQTL)** that regulate protein complex interactions, including **1,106 cis- and 1,740 trans-associations**. These findings offered **unprecedented insights into protein**

interactions beyond the resolution of standard bulk proteomics.-Through causal inference, we linked protein complexes to diseases such as **mesothelioma, heart disease, and hypothyroidism**, revealing protein complexes as potential biomarkers and therapeutic targets. This study established a novel approach in proteomics, paving the way for precision medicine and targeted interventions that leverage the network of protein interactions in human health and disease.

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P18.102.A 131 genetic loci highlight immunological pathways and tissues in asthma and nasal polyposis

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Background: Coexistence of asthma and chronic rhinosinusitis with nasal polyposis (CRSwNP) associate with allergic phenotypes, disease severity and failure of first-line treatment for both asthma and CRSwNP. Recent studies have highlighted shared genetic components for these diseases.

Material and Methods: To better understand this shared component, we performed genome-wide meta-analyses of asthma (n = 71,481), CRSwNP (n = 9626) and chronic rhinosinusitis without nasal polyposis (CRSsNP, n = 15,448) in FinnGen and UKB (685,602 controls).

Results: We detected 131 genomic associations, including 29 novel loci for asthma, 33 novel loci for CRSwNP, and one for CRSsNP. A shared impact to asthma and CRSwNP was observed at 62 loci. A cross-trait meta-analysis using all disorders further implicated 17 loci associating with asthma or asthma and CRSwNP. These loci largely cluster into three groups, with a shared risk being most frequent. We also find 12 nonsynonymous associating variants, including a novel *TP63* missense variant linked with CRSwNP (OR = 1.519 [1.331–1.734]). Gene set analyses confirmed enrichment of type 2 inflammation genes, and linked *FOXP3* signaling to CRSwNP.

Conclusion: Our results highlight new shared and separate genetic pathways for CRSwNP and asthma. These provide several avenues of further investigation in functional and epidemiological follow-up, and evidence for immunological and non-immunological mechanisms behind both diseases.

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P18.104.C Cross-species transcriptome meta-analysis of anterior cruciate ligament rupture

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Background: The Anterior Cruciate Ligament (ACL) plays a critical role in maintaining the musculoskeletal stability of the knee. Its injury has been linked to an increased risk of developing osteoarthritis. This study aims to identify cross-species responses to ACL rupture providing insights on its molecular basis.

Material and Methods: We analyzed five publicly available transcriptomic datasets from *Homo sapiens*, *Mus musculus*, *Canis lupus familiaris*, and *Oryctolagus cuniculus*. Differential gene expression analysis was performed for each dataset, producing a genome-wide transcriptional signature of fold-change significance for individual genes. Stouffer's method was used to integrate the results, identifying genes significantly deregulated across all species. Additionally, gene-set enrichment analysis revealed pathways that were consistently upregulated or downregulated.

Results: A positive correlation in expression was observed between human and the other three species ($r^2 = 0.177–0.305$, $p \leq 2.7 \times 10^{-113}$), identifying 210 genes as the most consistently up- and down-regulated in response to ACL rupture ($p\text{-adjusted} \leq 1.27 \times 10^{-23}$). These genes are primarily involved in cellular mitosis, collagen pathways, and cartilage development. Furthermore, 60 pathways were found to be significantly up- or down-regulated across all species ($p\text{-adjusted} \leq 4.57 \times 10^{-4}$). Among these, the upregulation of inhibition of bone mineralization ($p\text{-adjusted} \leq 2.99 \times 10^{-6}$) aligns with previous findings on reduction of subchondral bone mineral density following ACL rupture.

Conclusion: This study highlights that distinct species exhibit common molecular responses to ACL rupture, underscoring the value of mice, dogs, and rabbits as potential translational model organisms for ACL rupture research. Furthermore, the identified genes and pathways highlight the molecular mechanisms underlying ACL rupture.

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P18.105.D Multi-stage genome-wide association studies showed new insights to subarachnoid hemorrhage in the multi-ethnic populations

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Background: Subarachnoid hemorrhage (SAH) due to intracranial aneurysm (IA) rupture is a life-threatening condition with high mortality (up to 50%) and morbidity in the survivors (up to 30%). Previous genome-wide association studies (GWASs) have still limitations to explain a genetic heritability of SAH. Here, we performed a two-stage GWAS and meta-analysis to identify genetic susceptibilities to SAH in the large-scale populations.

Material and Methods: Two-stage Korean GWAS involved 151/175 SAH and 99/175 non-ruptured patients at discovery (2015–2019 years)/validation (2020–2023 years) stage, respectively, sharing 6,429,498 markers after a high-captured genotype-imputation process after pruning imputation score < 0.5. Independent GWASs collected 31/1539 SAH and 11/447 non-ruptured patients in Asian-/European-ancestry populations, respectively, from UK Biobank genotype-imputation data, sharing 93,095,623

markers. Genome-wide meta-analyses were performed by weighting each GWAS by the inverse variance approach.

Results: Nine variants within a serine/threonine-protein kinase 3 (*STK3*:8q22.2) gene showed a genome-wide significant association with SAH formation and complete linkage disequilibrium (LD) at the combined stage (OR = 0.46, $p = 4.51 \times 10^{-8}$), dramatically replicated at discovery and validation stages ($p = 0.0456$ and 2.76×10^{-7}, respectively). Twenty-seven of gene regions showed a suggestive association with SAH formation ($p < 1 \times 10^{-5}$), including two large LD structures such as *SLC2A13-LRRK2* (12q12) and *CCL15-CCL14* (17q12). None of any loci improved an association in either Asian- or European-ancestry GWAS meta-analyses including Korean GWAS results.

Conclusion: Our study shows novel insight modifiers in *STK3* susceptibility to SAH in the large-scale samples. Our findings will be warranted to validate in an independent population, thereby understanding genomic aspect to the pathology and etiology of SAH.

C.O.I. Disclosure:

E. Hong: None.

P18.106.A Shared Genetic Variants Linking ALS, Sporadic FTD, and Cognitive Traits

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Background: Cognitive impairment, including deficits in executive function, verbal fluency, and social cognition, affects ~50% of ALS patients with ~15% meeting criteria for frontotemporal dementia (FTD). The genetic basis of cognitive decline in ALS remains poorly understood. This study investigates shared genetic factors underlying ALS, sporadic FTD (sFTD), and cognitive traits to uncover biological mechanisms.

Material and Methods: Multi-trait GWAS analysis (MTAG) was conducted using European ancestry data for ALS (n = 138,086), sFTD (n = 19,993), and cognitive traits, including cognitive performance (n = 257,828), executive function (n = 78,547-11,263), fluid intelligence (n = 171,304), and memory (n = 331,679). Shared loci were validated using conjunctional FDR (conjFDR), and local genetic correlations between ALS and sFTD were assessed via LAVA.

Results: ALS showed significant negative genetic correlations with cognitive traits, including cognitive performance (CP) ($r = -0.19$, $p = 4.85 \times 10^{-6}$) and fluid intelligence ($r = -0.20$, $p = 2.74 \times 10^{-6}$). MTAG for ALS boosted by CP identified novel associations in NEGR1, CLCN3, MEF2C, EXOC4, TSNARE1, and EFL1/SAXO2. ConjFDR validated findings for MEF2C, EXOC4, and EFL1/SAXO2, and revealed MYO19 as a new locus. Gene set annotation implicated Mecp2-mediated gene activation. LAVA detected two significant local genetic correlations between ALS and sFTD, involving ERGIC1 (chromosome 5) and SLC9A8/SPATA2 (chromosome 20).

Conclusion: This study highlights shared genetic architecture between ALS, sFTD, and cognitive traits, with 7 novel risk genes identified. While local genetic correlations confirmed prior findings, limitations in sFTD GWAS design and sample size restricted discovery of additional loci.

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P18.107.B Genetic mapping of lifespan and mitochondrial stress response in *C. elegans*

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Background: The mitochondrial unfolded protein response (UPRmt) is one of the mito-nuclear regulatory circuits that can restore mitochondrial function upon a variety of stress conditions, improving metabolic health and extending lifespan. However, the complex gene interactions that influence this pathway and its relationship to aging and healthspan remain to be elucidated.

Material and Methods: Doxycycline (Dox) was applied to trigger mitochondrial stress in a worm genetic reference population with 85 *C. elegans* recombinant inbred advanced intercross lines (RIAILs). In this study, we assessed molecular profiles—transcriptome, proteome, metabolome, and lipidome—and life-history traits, including lifespan, development, growth dynamics, and reproduction.

Results: Here, we found that Dox-induced lifespan extension exhibited large variations within 85 strains. Using multi-omics integration, aging molecular signature was also uncovered, which can be partially reversed by Dox. To indicate the mechanisms underlying Dox-induced lifespan extension, we applied quantitative trait locus analyses and found one UPRmt modulator, *fipp-1/FIP1L1*, which was validated in *C.elegans* and humans. In human UK biobank, *FIP1L1* was also associated with metabolic homeostasis.

Conclusion: Overall, our findings demonstrate a novel UPRmt modulator across species and provide insights into potential translational research.

C.O.I. Disclosure:

X. Li: None.

P18.108.C Decoding diversity in disease: a novel approach to scoring genes in polygenic and rare diseases for diagnostic insights

Aleksandra Suwalska[1], *Karolina Kulis*[1], *Walter Wolfsberger*[2], *Khrystyna Shchubelka*[2], *Ana Topf*[3], *Marie Vandroux*[4], *Sara Pini*[5], *Marco Savarese*[6], *Jocelyn Laporte*[4], *Volker Straub*[3], *Filippo M. Santorelli*[7], *Rossella Tupler*[5], *Taras Oleksyk*[2], *Joanna Polanska*[1]

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Background: The diagnosis of numerous polygenic or rare conditions remains a significant challenge due to their complex and understudied aetiology. There is an urgent requirement to develop sophisticated computational tools that will facilitate understanding of the underlying molecular mechanisms.

Material and Methods: A gene scoring system was developed to quantitatively assess the impact of genomic variants, enabling the assessment of their contribution to diseases with unclear aetiology. The effectiveness of the gene scoring system was then tested on WES datasets from polygenic (type 1 diabetes T1DM, 2667 patients and 4529 controls, an ethnically homogenous

Ukrainian cohort) and rare diseases (neuromuscular diseases NMD, 884 patients and 70 controls from the British population), with scores compared using the Mann-Whitney U test and effect size calculated as rank biserial correlation coefficient. Gene set enrichment analysis (GSEA) was then performed, with genes being ranked based on the effect size.

Results: Genes with at least a small effect size were identified as associated with the studied diseases. For T1DM, the top genes included HLA-DRB5, HLA-DRB1, HLA-DQA1, HLA-DQB1, and LTA. The significantly highlighted pathways were strongly linked to immune response and T1DM development. In NMDs, the top enriched pathways include the extracellular matrix organisation, Notch signalling, and intracellular signalling by second messengers.

Conclusion: The proposed gene scoring effectively identifies critical genomic variations and associated pathways in both polygenic and rare diseases. The approach provides valuable insights into the molecular mechanisms, highlighting its potential to support accurate diagnosis and further research into disease pathogenesis.

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P18.109.D Genome-wide association study identifies novel risk loci for Inclusion Body Myositis

Nathan Routledge[1], *Pedro Machado*[1], *Jana Vandrovcova*[2], *Sara Nagy*[3], *MYOGEN Consortium*[4], *Janine Lamb*[5], *Michael Sinnreich*[3], *Tom Lloyd*[6], *Damien Amelin*[7], *Chris Conrad*[8], *Ambreen Tariq*[9], *Melissa Hayes*[10], *Brianna Blume*[11], *Heidi Runk*[12], *Perry Shieh*[13], *Angelini Corrado*[14], *Chrysa Chrysovitsanou*[15], *Marinos Dalakas*[16], *Lorenzo Maggi*[17], *Linda Carter*[18], *Anders Oldfors*[19], *Christina Liang*[20], *Amy Bartlett*[21], *John Kissel*[21], *Merrilee Needham*[22], *Anthony Amato*[23], *James Miller*[24], *Anne Ankers*[24], *Willem De Ridder*[25], *Edmar Zanoteli*[26], *Tahseen Mozaffar*[27], *Dawn Caraballo*[28], *Jeffrey Guptill*[29], *Boel de Paepe*[30], *Stefan Brady*[31], *Olimpia Carbunar*[32], *Miren Zulaica*[33], *Diego Lopergolo*[34], *Elie Naddaf*[35], *Henry Houlden*[1]

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Background: Inclusion Body Myositis (IBM) is a subtype of the heterogeneous idiopathic inflammatory myopathies (IIM) autoimmune disorders. IBM has a complex aetiology with a combination of inflammatory and degenerative aspects. This study was performed to be the first large scale genome-wide association study of patients with IBM.

Material and Methods: We analysed 1036 Caucasian IBM patient samples collected via the International Centre for Genomic Medicine in Neuromuscular Diseases framework and matched with 4240 samples from the Wellcome Trust Case Control Consortium (WTCCC) and the National Blood Survey cohort. Following quality control and imputation, single variant association testing was conducted using the Scalable and Accurate Implementation of Generalized Mixed Model (SAIGE). Further downstream analysis tests such as gene-set enrichment, pathway-analysis, co-localisation, and functional prediction were also performed.

Results: As with previous IIM GWAS studies the HLA locus DRB1 region was the most associated. A further four novel loci non-HLA loci passed genome-wide significance: AHRR, CD200, RMI2, and ORMDL3, as well as previous IIM associated loci PTPN22 and SH2B3/ATXN2. Further analysis found significant association with the genes SCHIP1, GSDMB and PDCD6. Functional annotation also found enrichment of the lead SNPs within regulatory elements in muscle and immune cell types.

Conclusion: We found strong associations and novel loci in IBM in muscular and immune cell types for both autoimmune and neurodegenerative pathways.

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P18.110.A Data-processing pipeline to find and evaluate possible associations of tandem repeat alleles with ulcerative colitis

Ingrid Lojova[1,2,3], *Andrej Balaz*[1,4], *Marcel Kucharik*[1,2,4], *Jaroslav Minarik*[1,3], *Svatopluk Puterka*[5], *Juraj Gazdarica*[2,4,6], *Andrea Zatkova*[1], *Tatiana Sedlackova*[2,4], *Tomas Szemes*[2,3,4], *Jaroslav Budis*[2,4], *Jan Radvanszky*[1,2,3]

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Background: Complex diseases are influenced by several factors, including tens/hundreds of different genomic elements. Although conventional GWAS (genome-wide association studies) already identified SNVs (single nucleotide variants) linked to many diseases, tandem repeats (TRs) seem to contribute to disease susceptibility significantly. Fine mapping of TRs near already associated SNVs, therefore, offers a possibility to study genomic determination. We characterized TR motif variability in genomic loci associated with ulcerative colitis (UC).

Material and Methods: Our pipeline was developed and validated using a whole-genome sequencing dataset of 17 UC patients and 109 controls. It integrates in-house applications, including PolyRisk to define relevant SNVs, a python script to identify TR motifs within ±25 kbp of each SNV, Dante for TR genotyping, visualization and statistical comparisons.

Results: Based on specific filtering of GWAS-reported associations and polygenic score calculations, we selected 135 SNVs potentially relevant to UC in our patients. Within their ±25 kbp regions, we found 2015 TR motifs and characterized their nucleotide-scale variability and distribution patterns.

Conclusion: We have created a pipeline to find associations of TR alleles with UC. We found that TRs near UC-associated SNVs are variable, even with certain distribution differences between the studied groups. These loci may influence disease susceptibility, but further validation with larger cohorts and automated analyses is needed. Expanding this approach to additional complex diseases may enhance understanding of their genetic architecture.

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P18.111.B The light within: architecture, health, circadian rhythms, and the power of omic science

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Background: In the context of anthropization of today, it is well-established that architectural design significantly affects the health and well-being of people living in cities.

Material and Methods: A comprehensive literature review was conducted by analyzing recent studies through academic databases to identify research exploring the relationship between architectural design, light exposure, and health outcomes, with a particular focus on circadian rhythm disruption.

Results: Our search revealed that Light is a powerful external signal for the circadian system. Its detection involves three types of photoreceptors in the retina particularly the specific type of ganglion cell (ipRGCs) that expresses a photosensitive pigment called melanopsin (Opn4), making them intrinsically photosensitive. IpRGCs are not involved in image perception but in the detection of ambient light influencing the circadian rhythm. Epidemiological studies have linked circadian disruption to an increased susceptibility to many diseases such as cancers. Studies exploring the connection between architecture, light, and circadian biology are rare. A recent study used a genome-wide gene-by-environment approach to examine how light affects genes linked to chronotype and circadian rhythms. It identified a light sensitivity locus in the ARL14EP gene, showing a genetic correlation between light sensitivity, a later chronotype, and an increased risk of PTSD and insomnia.

Conclusion: It appears that architectural design can affect the circadian biology of individuals by influencing their exposure to light, but further and more innovative research, such as using circadian transcriptional profiles and genome-wide gene-by-environment interaction studies, is needed.

C.O.I. Disclosure:

N.B. Abdelmoula: None. **B. Abdelmoula:** None. **E. Abdelmoula:** None.

P18.112.C Exploring the role of maternal epigenetic drift in spontaneous preterm birth

***Davide Sacco**[1,2], Luciano Calzari[3], Anna Maria Di Blasio[4], Giulia Zangheri[5], Fulvio Ferrara[6], Lucy Costantino[2], Michele Vignali[5], Davide Gentilini[1,3]*

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Background: Recent studies suggest maternal DNA methylation may influence preterm birth. However, epigenetic drift, defined as the accumulation of sporadic aberrations in the methylome, termed stochastic epigenetic mutations (SEMs), remains under-explored in maternal blood and its correlation with offspring.

Material and Methods: This study analyzed 35 mother-child pairs (PTB n = 18, Fullterm n = 17) using the Illumina Methylation 450k Array from maternal and cord blood. SEMs are extreme DNA methylation outlier values. Once identified, we used a logistic model to evaluate the global epigenetic drift between PTB and full-term groups, and SKAT test for gene-level drift.

Results: Maternal hypomethylated SEMs were significantly associated with PTB (FDR = 0.02, OR = 1.042, 95% CI: 1.017–1.084), indicating a 4.2% higher likelihood of PTB per unit increase in SEMs burden. SKAT identified 367 maternal PTB-associated genes, enriched in the focal adhesion pathway. In offspring, hypomethylated epigenetic drift was also higher in PTB infants, with SKAT identifying 45 genes, including KRT1 and TESK1, shared with maternal results.

Conclusion: Hypomethylated drift may contribute to PTB onset through gene alterations in the focal adhesion pathway, and disrupting this pathway can be associated with preterm labor. The shared drift in offspring could suggest a potential inheritance mechanism, predisposing preterm infants to long-term health risks. For instance, KRT1, a gene associated with keratinocyte-related diseases in newborns, shows evidence of maternal drift, suggesting that this shared drift could increase susceptibility to these conditions in preterm infants. Further studies with larger sample sizes are necessary to validate these findings.

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P18.113.D Haplotype analysis identifies functional elements in MGUS

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Genome-wide association studies (GWASs) based on common single nucleotide polymorphisms (SNPs) have identified several loci associated with the risk of monoclonal gammopathy of unknown significance (MGUS), a precursor condition for multiple myeloma (MM). We hypothesized that analyzing haplotypes might be more useful than analyzing individual SNPs, as it could identify functional chromosomal units that collectively contribute to MGUS risk. To test this hypothesis, we used data from our previous GWAS on 992 MGUS cases and 2910 controls from three European populations. We identified 23 haplotypes that were associated with the risk of MGUS at the genome-wide significance level ($p < 5 \times 10-8$) and showed consistent results among all three populations. In 10 genomic regions, strong promoter, enhancer and regulatory element-related histone marks and their connections to target genes as well as genome segmentation data supported the importance of these regions in MGUS susceptibility. Several associated haplotypes affected pathways important for MM cell survival such as ubiquitin-proteasome system (RNF186, OTUD3), PI3K/AKT/mTOR (HINT3), innate immunity (SEC14L1, ZBP1), cell death regulation (BID) and NOTCH signaling (RBPJ). These pathways are currently important therapeutic targets for MM, which may highlight the advantage of the haplotype approach homing to functional units.

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P18.114.A Lessons learnt from GWAS and single cell RNA-seq data integration methods

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Background: Genome-wide association studies (GWAS) and single cell RNA-seq provide essential data for genetic cell-type mapping. Integrating these data enables understanding genetic variation at the cell-type level. However, no standards exist for evaluating methods that infer how genetic variation influences traits using cell-type-resolved transcriptomes.

Material and Methods: We categorize existing methods into two strategies (Table 1). We establish a benchmark for systematic evaluation with 12 scRNA-seq datasets and 35 GWAS datasets. We validate results with an independent brain data with the same individuals based scRNA-seq and scATAC-seq data spanning six human brain developmental stages.

Results and Conclusion: 1) "Cepo" is the superior method for defining cell-type specificity genes in genetic inference of cell type-trait/disease associations, though differential expression tests best rank gold-standard marker genes. 2) Performance is comparable with mouse- or human-derived transcriptomic data. 3) Method power increases with GWAS sample size and cell count per type. 4) We introduce a Cauchy p-value combination method integrating both strategies maximizes power while avoiding false positives. 5) Single-cell atlases or diverse single-organ datasets yield greater power than those with limited cell-type representation. 6) scRNA-seq data covering various brain developmental stages and stage-specific cell types enhance identification of cellular contributors to brain disorders and cognitive traits. 7) The superior performance of the Cauchy combination was validated with independent scATAC-seq data from the same individuals. 8) Leveraging schizophrenia-associated cell types significantly identified via GWAS and scRNA/ATAC-seq annotations improves polygenic risk prediction for schizophrenia.

Analytic strategies of different methods

Strategy	Method	Framework
From single cell to GWAS	e.g. sLDSC, MAGMA-gene set, sc-linker, CELLECT etc.	Takes cell-type-specific annotations in heritability enrichment analyses
From GWAS to single cell	e.g. scDRS, scPagwas	Uses GWAS-derived gene lists to make single cell disease risk scores based on cumulative scRNA-seq expression of the genes

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P18.115.B Genetic associations with endometriosis and its sub-phenotypes: Insights from a multicenter Italian GWAS

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Background: Endometriosis is a complex disease characterized by different symptomatic manifestations and influenced by various genetic and environmental factors. The largest meta-analysis to date (Rahmioglu et al., 2023), identified 42 risk loci associated with endometriosis and its sub-phenotypes. Our study aims to investigate genetic factors associated with endometriosis and its sub-phenotypes within the Italian population; therefore, we conducted a Genome-wide association study (GWAS).

Material and Methods: This multicenter study involved specialized Italian centers that collaborated in collecting DNA samples from patients with surgically diagnosed and histologically confirmed endometriosis. We analyzed genotyping data from 862 cases and 1843 controls. SNP associations with endometriosis were evaluated, including analyses of 10 distinct phenotypic manifestations of the disease. Association analysis was conducted on the post-imputation SNPs dataset using a logistic regression model adjusted for covariates.

Results: Several polymorphisms were associated with endometriosis and its sub-phenotypes (raw p-value $<10^{-5}$). Notably, two SNPs reached genome-wide significance after correction for multiple testing (FDR < 0.05) in the gastrointestinal pain sub-phenotype analysis: rs185338542, located upstream of the ACOT7 gene, and rs138188726, an intronic variant in PCDH7. Additionally, 13 SNPs nominally significant in our Italian cohort overlapped with findings from the latest meta-analysis, supporting their role in endometriosis susceptibility.

Conclusion: Analyzing sub-phenotypes improves the genetic characterization of endometriosis. These results suggest that common variants may contribute to symptom heterogeneity, indicating that polymorphisms can have differentiated effects across the sub-phenotypes of the disease. Finally, our results were found to be consistent with those of the latest published meta-analysis.

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P18.116.C Exploring the molecular basis of the genetic correlation between body mass index and brain morphological traits

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Background: Several studies have demonstrated significant phenotypic and genetic correlations between body mass index (BMI) and brain morphological traits derived from structural magnetic resonance imaging (sMRI). While there is evidence that increased BMI is associated with reduced gray matter volume, the related neurobiological mechanisms have yet to be characterized.

Material and Methods: Using LD score regression we estimated the genetic correlation between BMI and 435 brain morphology traits using genome wide association studies summary data from the UK BioBank. We then used summary-based Mendelian randomization (SMR) to identify genes whose genetically regulated expression (GReX) in 13 brain tissues from GTEx pleiotropically affects BMI and brain morphology. Finally, we used fine-mapping and colocalization (coloc-SuSie) to identify cases in which a single variant is associated with both BMI and brain morphology through gene expression.

Results: In agreement with previous literature, we find many sMRI traits to have negative genetic correlation with BMI, including in particular cortical areas, cortical and subcortical volumes. SMR analysis found 21 genes whose GReX pleiotropically affects brain morphology and BMI. Fine-mapping followed by colocalization analysis allowed, in several cases, the identification of credible sets of variants likely to be causal for both the macroscopic phenotypes and for gene expression. This happens for protein-coding genes VPS11 and TUFM, and antisense transcripts RP11-624M8.1 and RP11-552F3.9.

Conclusion: We have shown how the integration of data allows the molecular dissection of the genetic correlation between complex traits and the formulation of data-driven hypotheses on the relevant regulatory mechanisms.

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P18.117.D A genetic atlas of protein complexes in 200 adults from the Shetland Isles

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Protein complexes play critical roles in cellular processes, yet their genetic basis remains poorly understood. Here, we inferred 2,175 protein complex phenotypes based on a deconvolution technology and conducted GWAS in 200 individuals from the Shetland Isles. We identified 321 genome-wide significant independent protein complex quantitative trait loci (pcQTL), including 196 *cis*-pcQTL ($P < 5 \times 10^{-8}$) and 125 *trans*-pcQTL ($P < 2.3 \times 10^{-11}$). Among these, 17 *cis*-pcQTL were novel, while 85 *cis*-pcQTL had stronger effects compared to the conventional pQTL from GWAS of single proteins. We discovered 970 colocalized associations between protein complexes and human complex traits, where 959 showed significant potential causal effects between 143 protein complexes and 156 traits (FDR < 5%). We systematically investigated the protein complex markers identified for their therapeutic potential. Among the 190 protein complexes (covering 37 protein subunits) with *cis*-pcQTL, 51 protein complexes (covering 11 protein subunits) showed potential causal effects for at least one disease or health-related phenotype (FDR < 5%). Based on the DrugBank and Drugs.com databases, we found 37 drugs targeting 5 of the 11 protein subunits, resulting in 226 drug-complex-disease combinations. In summary, we provided valuable insights into the genetic architecture of protein complexes and identified new therapeutic possibilities between protein complexes and disease risk hidden in traditional analyses.

C.O.I. Disclosure:

J. Chen: None. **X. Shen:** None.

P18.118.A Investigating the shared genetic aetiology of hypertension and type 2 diabetes

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Background: Hypertension and type 2 diabetes (T2D) are common long-term conditions (LTCs) that frequently co-exist. We aimed to utilise statistical genetic approaches to uncover shared genetic mechanisms acting through pathways distinct to those of known risk factors.

Material and Methods: We estimated the genetic correlation between hypertension and T2D using LD-score regression and investigated the bidirectional causal relationship using Mendelian randomisation (MR). We quantified the contribution of 10 common risk factors in explaining the genetic correlation between hypertension and T2D and re-estimated the SNP-disease effects after 'removing' the causal effects of identified risk factors. We used colocalisation to identify shared causal genetic variants and investigated the genes and pathways implicated.

Results: Hypertension and T2D had strong genetic correlation ($rG = 0.48$, $p = 2*10^{-196}$), with evidence of a bidirectional causal relationship. BMI, waist-hip ratio, and triglycerides had the largest individual contributions to the genetic correlation. After removing the combined causal effects of these, substantial genetic correlation persisted ($rG = 0.31$, $p = 2*10^{-36}$), indicating remaining shared mechanisms. Sixteen regions showed evidence of a shared causal variant, including an intronic variant in *PCSK7*, previously associated with iron homeostasis, and pQTLs for NCF1, linked to oxidative stress; evidence from DrugBank supports this protein as a potential drug target.

Conclusion: We identified shared genetic causes between hypertension and T2D independent of well-known factors such as BMI; this increased understanding may be useful in future treatment and prevention of this key LTC pair.

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P18.120.C Understanding the common genetic architecture of spontaneous coronary artery dissection using genetically-related phenotypes

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Background: Spontaneous coronary artery dissection (SCAD) is significant cause of heart attacks and disproportionately affects women, but remains poorly understood. Genetic variants have been implicated in SCAD, and a recent genome-wide association study (GWAS) highlighted genetic overlap with other traits and high heritability - most of which is unidentified. Here, we aim to leverage the known genetic architecture of traits associated with SCAD to better understand its genetic risk via a multi-trait GWAS, followed by polygenic risk score (PRS)-based validation.

Material and Methods: A multi-trait analysis of GWAS (MTAG) was performed using published GWASs of SCAD and three genetically correlated traits: fibromuscular dysplasia, migraine, and blood pressure. Independent SCAD-MTAG variants of genome-wide significance were applied as a PRS on a cohort of $N = 105$ SCAD patients and $N = 67$ controls. The association of the SCAD-PRS and SCAD status was assessed via logistic regression, while controlling for sex and genetically determined principal components.

Results: Multi-trait GWAS analysis of SCAD and related traits reinforces the shared genetic aetiology of these traits, particularly between blood pressure and SCAD. This approach identified 19 loci, of which two were novel, and which may contribute to the extreme sex bias observed in this condition. PRS analysis of these results showed a significant relationship between these results and SCAD status (OR = 1.99, 95% CI = 1.28–3.08, p-value = 0.002).

Conclusion: Using related traits, we have identified additional common variants associated with SCAD and demonstrated the usefulness of this method via the production of a PRS with a strong relationship to disease.

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P18.121.D Germline structural variants as risk alleles for high grade serous ovarian cancer

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Background: Majority of women suffering from epithelial ovarian cancer (EOC) are diagnosed at stages III-IV with an aggressive histotype, high-grade serous ovarian cancer (HGSOC), and <30% of women survive past 5-years. Women inheriting high-moderately penetrant rare variants in genes of the homologous recombination repair pathway account for ~15–20% of cases. The rest harbor a combination of common risk variants identified from genome-wide association studies (GWAS) that individually confer small amounts of risk but collectively contribute to a complex polygenic architecture. Disease risk conferred through single nucleotide polymorphisms (SNPs) accounts for ~40% of the total sum of risk variants, with the majority of variants awaiting identification. Increasing the compendium of risk variants will aid women who can leverage the improved outcomes at earlier stages of diagnosis but are presently unidentifiable.

Material and Methods: We hypothesized that structural variants (SVs), which encompass a greater proportion of DNA than sequence variation via SNPs, confer risk for EOC and HGSOC. We performed a preliminary analysis in Genomics England where SVs were identified from whole-genome sequencing (WGS) data using the Manta-Canvas pipeline.

Results: We identified 3 variants impacting copy number (CNVs) that are presently at a significantly different frequency in 586 EOC cases compared to 2000 age, sex and ethnicity matched control participants ($p < 0.05$). We are expanding the set of cases to include those in the UK Biobank, All of Us and newly generated internal datasets.

Conclusion: The results of this analysis indicate germline regions associated with EOC heritability due to structural variation.

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P18.122.A Genome wide association study meta analysis of detoxification and oxidative stress related genetic variants in prostate cancer

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Background: Prostate cancer (PCa) is a multifactorial disease influenced by genetic and environmental factors. While genome-wide association studies (GWAS) have identified multiple variants associated with PCa risk, the role of genes involved in detoxification, oxidative stress, and DNA repair remains incompletely understood. This study conducted a GWAS meta-analysis to identify genetic variants in these biological processes and evaluate their impact on PCa susceptibility.

Material and Methods: A comprehensive search of the literature and genomic databases, including GeneCards and PubMed, identified 279 genes involved in detoxification, oxidative stress, and DNA repair. Summary data from the PRACTICAL Consortium meta-analysis (79,148 PCa cases, 61,106 controls) were used to detect potential risk variants. A meta-analysis was performed using GWAS data from the FinnGen and UK Biobank cohorts (19,209 PCa cases, 288,847 controls). Variants were selected based on meta-analysis p-value < 0.01, minor allele frequency > 10%, and relevance to detoxification, oxidative stress, and DNA repair pathways.

Results: Summary data from the PRACTICAL Consortium meta-analysis identified 1,287 significant variants in 167 genes. Following the meta-analysis, 258 SNPs in 30 genes were significantly associated with PCa. These variants were prioritized based on linkage disequilibrium ($r^2 \geq 0.8$, $D' \geq 0.9$), p-value < 0.01, and involvement in pathways related to detoxification, oxidative stress, and DNA repair.

Conclusion: This study highlights the role of detoxification and oxidative stress related genes in PCa susceptibility and suggests that the identified variants could serve as biomarkers for risk and aggressiveness assessment. Further validation studies are needed to confirm these findings.

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P18.123.B Association Analysis of Small Vessel Disease Variants Identifies a Novel *CTC1* Variant Linked to Migraine Susceptibility

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Background: Migraine is a common neurological disorder affecting 15% of the global population. While vascular factors and genetic components are implicated in its pathogenesis, the relationship between migraine and cerebral small vessel disease (SVD) remains unclear. This study investigated the association between SVD-related genetic variants and migraine susceptibility.

Material and Methods: We conducted an association analysis of 37 single nucleotide polymorphisms (SNPs) in a cohort of 548 Caucasian individuals (267 migraine cases, 281 controls). SNPs were genotyped using a custom Sequenom MassARRAY assay and High Resolution Melt analysis. After quality control including Hardy-Weinberg equilibrium testing and linkage disequilibrium analysis, multiple testing corrections were applied using various methods including Bonferroni, Holm-Bonferroni, SIDAK, and Benjamini-Hochberg procedures.

Results: The missense variant rs62624978 (p.Gly414Val) in the CTC1 gene showed significant association with migraine across all correction methods ($p < 0.05$). Additionally, rs6478241 in ASTN2 demonstrated marginal association, though only remaining significant under false discovery rate correction. The CTC1 variant represents a novel finding in migraine genetics, potentially linking telomere maintenance and vascular integrity to migraine susceptibility.

Conclusion: Our findings identify CTC1 as a novel genetic factor in migraine susceptibility, suggesting a potential role for vascular integrity and telomere maintenance in migraine pathophysiology. This discovery provides new insights into the vascular contributions to migraine and highlights the genetic overlap between migraine and small vessel disease. Further research in larger, diverse populations is needed to validate these findings and explore their functional implications.

C.O.I. Disclosure:

Z. Molaee: None.

P18.124.C Genetic variant in*HMCN2*alters ETS-binding and impacts periodontitis risk

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Background: Single nucleotide variants (SNVs) in the Hemicentin-2 (HMCN2) gene are associated with increased risk for stage III-IV Grade C periodontitis in young adults ≤35 years of age ($p = 6.1 \times 10^{-8}$). HMCN2, a key component of the collagen-containing extracellular matrix, maintains tissue structural integrity. This study aimed to identify the causal SNV and its biological consequences.

Material and Methods: SNVs were analyzed for transcription factor (TF) binding site alterations using the TF Affinity Prediction Tool (TRAP). Functional effects were assessed via Electrophoretic Mobility Shift Assay(EMSA) and allele-specific reporter gene assays. HMCN2 expression in gingival cells and biopsies was evaluated by qRT-PCR, immunohistochemistry, and immunofluorescence.

Results: SNV rs115281761 resided in a predicted binding site of the TF ETS Proto-Oncogene 2 (ETS2), and its rare G-allele ($MAF = 0.01$) altered the GGAA/T core motif ($P < 0.0001$). ETS2 is specifically expressed in basal epithelial cells and monocytes. EMSA confirmed ETS2 binding at rs115281761 and showed that the G-allele significantly reduced binding affinity. Reporter assays revealed that the region acts as an enhancer (3x induction, $p < 0.001$), with the G-allele reducing activity by 47% compared to the non-effect C-allele. HMCN2 expression was higher in gingival epithelial cells (9.5 fold) than in fibroblasts, with protein mainly localized to the epithelial basal membrane.

Conclusion: SNV rs115281761 in HMCN2 likely contributes to stage III-IV grade C periodontitis by altering ETS2 binding and HMCN2 enhancer activity, compromising gingival tissue integrity. These findings provide mechanistic insights into the genetic basis of severe periodontitis.

C.O.I. Disclosure:

M. niu: None. **A. Schaefer:** None.

P18.126.A Omic study of intermediate phenotypes in colorectal cancer

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Background: Colorectal cancer (CRC) is a major global health challenge with complex genetic underpinnings. While Genome-Wide Association Studies (GWAS) have identified over 200 loci linked to CRC risk, limited sample sizes leave hereditary factors incompletely understood. Telomere length (TL) provides a framework for exploring molecular phenotypes and disease complexity.

Material and Methods: We conducted a telomere length association study (TL-WAS), using TL as an intermediate molecular phenotype. Blood ($n = 51$) and colonic mucosa ($n = 76$) samples were used to model genetically predicted TL and assess its association with CRC risk. For TL-QTL mapping, telomeres were randomly assigned chromosomal positions, and the genome was divided into 1 Mb bins. TL-QTLs were identified by correlating TL with genetic variants. Using Nested Cross-Validated Elastic-Net, SNPs were assigned weights for TL prediction, and significant models were retained. MetaXcan integrated GWAS data with prediction models to evaluate gene-level associations between predicted gene expression and CRC risk.

Results: Using Nested Cross-Validated Elastic-Net, we identified 4,850 TL-QTLs in colonic mucosa and 4,195 in blood, with each variant assigned a weight reflecting its association with TL. These TL-QTLs were subsequently used in the CRC association study. Our analysis revealed a significant association between TL and CRC risk in both colonic mucosa ($p = 0.0051$) and blood ($p = 0.0181$).

Conclusion: Our study suggests that TL may be a useful biomarker for CRC. We also developed a model adaptable to other omics data. This approach may help identify new risk pathways and biological insights, improving resource allocation in population screening for individuals at higher CRC risk.

C.O.I. Disclosure:

C. Macias Real: None. **S. Carlés González:** None. **I. Tomlinson:** None. **C. Fernández Rozadilla:** None.

P18.127.B Exploring the association between genetic variants and the risk of developing type 2 diabetes in Uzbekistan

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Background: Type 2 Diabetes (T2D) affects nearly half a billion people worldwide, yet genetic studies largely exclude Central Asian populations. This study investigates T2D-associated genetic variants in Uzbeks, aiming to enhance the understanding of T2D genetics and underscore the importance of including diverse populations in research to improve global disease models and precision medicine.

Material and Methods: This study encompassed 96 patients diagnosed with T2D and 96 healthy individuals of Uzbek ethnicity. Genetic screening was performed using the Global Screening Array v3.0, and statistical analyses were conducted using SNPassoc to examine genotype-disease associations under common genetic models.

Results: Under the additive model, several variants showed significant associations with T2D. Protective effects were found for rs4660329 (*MAST2*, $p = 1.91 \times 10^{-5}$, $OR = 0.40$), rs5909292 (*MAP3K15*, $p = 1 \times 10^{-4}$, $OR = 0.24$), and rs5909301 (*MAP3K15*, $p = 1.6 \times 10^{-4}$, $OR = 0.36$). Conversely, increased T2D risk was associated with rs16872304 (*ZFPM2*, $p = 3.30 \times 10^{-5}$, $OR = 3.56$), rs3859563 (*FUT6*, $p = 2.25 \times 10^{-4}$, $OR = 2.08$), and rs11002834 (*ZMIZ1*, $p = 5.43 \times 10^{-4}$, $OR = 3.43$).

Conclusion: Variants in *MAST2*, *ZFPM2*, *MAP3K15*, and *ZMIZ1* have been associated with T2D in previous studies, as documented in the GWAS catalog, highlighting their potential role in disease susceptibility and warrant further investigation to understand their biological significance.

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P18.129.D A generic polygenic score for preeclampsia and tailored models exhibit similarly low discriminatory power

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Background: This work developed a new "generic" Polygenic Risk Score for the prediction of preeclampsia based on maternal UK-Biobank (UKB) data following established protocols and compared it to a systematic review of the literature

Material and Methods: Using UKB raw data and following published protocols and public tools (PLINK 1.9, PRSice-2) we produced a Genomic Wide Association Studies (GWAS) and Polygenic Risk Score (PRS) model for PET vs. healthy controls using UKB target and validation cohorts. Following PRISMA 2020 guidelines a literature search was conducted (March 2024). Quality assessment and comparison were conducted using modified Polygenic Risk Score Report Standards 2021 (PRS-RS).

Results: Our model achieved a ROC $AUC = 0.57$, increased OR from 1.2 to 4 for the top 1% compared to 25% quartile. The systematic review included 7 PET-PRS reports using LDpred2, ORS-CS, and machine learning methods. Four of the studies generated GWAS summary data; while the remaining three used published GWAS summaries from hypertension datasets without PET population. Reported PET-PRS ROC-AUC and C-statistics $AUC = 0.62$, 0.64, and 0.649.

Conclusion: Reported PRS methods and endpoints differ significantly precluding meaningful metanalysis. The ROC-AUC reported, including ours, were in the low discriminatory zone. The limited success of all the models regardless PRS methodology, suggests that the maternal genetic component of PET is limited. Future modes may benefit from integrating paternal and fetal genomic data and additional non-genetic factors.

C.O.I. Disclosure:

A.L. Zimerman: None. **T. Reich:** None. **O. Reish:** None. **R. Unger:** None.

P18.130.A Exploring female analogs of male-pattern hair loss

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Background: Male-pattern hair loss (MPHL) is a prevalent and male-specific phenotype with a strong genetic basis. Whether an analog exists in females has been debated, with traits such as female-pattern hair loss (FPHL) or polycystic ovary syndrome (PCOS) being theorized. Here, we explore female analogs to MPHL, leveraging a polygenic risk score (PRS) approach and LD score regression (LDSC) to test for associations between MPHL-related genetic risk factors and female-specific traits or medical conditions.

Methods: Using data from the UK Biobank, we constructed MPHL-PRS from 169,105 men and calculated MPHL-PRS for 197,495 women, which we regressed on medical conditions and female-specific traits. LDSC was performed between MPHL and 2,148 phenotypes using public summary statistics from the male and female UK Biobank populations, respectively. Additionally, we conducted LDSC between MPHL and FPHL (based on an unpublished in-house GWAS in Europeans).

Results: The analyses showed no significant associations or genetic correlations after correction for multiple testing. However, some trends were observed ($P < 0.01$), including positive associations with malignant skin neoplasms, hypopituitarism, hypothyroidism, and fewer live births. MPHL further showed genetic correlations with earlier puberty and higher bone mineral density in females, mirroring previous findings in males. No association or genetic correlation with PCOS or FPHL was identified.

Conclusion: Overall, the data suggest no clear female analog to MPHL, though the phenotype may be less pronounced, potentially encompassing both positive and negative health outcomes. Further research is needed to elucidate the relationship between genetic risk factors for MPHL and female-specific health outcomes.

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P18.131.B The genetic basis of the immune response to Sars-CoV-2 infection in the Italian municipality of Vo'

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Background: It is becoming increasingly evident that SARS-CoV-2 infection is here to stay. Therefore, understanding whether genetic variants may impact the response to the virus or vaccination is crucial. Studies on the genetics of the immune response to SARS-CoV-2 have been hampered by the lack of genetically homogenous populations and longitudinal studies, assessing the response to infection and vaccination through the lens of the individual genetic makeup.

Material and Methods: Here we conducted genotyping and whole-genome sequencing of a well-annotated and closely followed population, the municipality of Vo', that has provided crucial insights on SARS-CoV-2 infection and COVID-19 clinical manifestations.

Results: We identified MHC variants associated with altered dynamics of the T cell response to infection. Additionally, when examining the response to vaccination, we identified MHC genetic variants that correlated with either increased or decreased TCR responses. We generated a polygenic risk score able to comprehensively capture these variations. Finally, we modelled some of these variants altering HLA proteins and identified the aminoacids most likely impacting their interactions with SARS-CoV-2 epitopes.

Conclusion: In all, we provide robust indications of genetic profiles modulating the immune response in a longitudinal study that may provide important clues and directions in designing appropriate public health interventions.

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P18.132.C Genetic variants in surfactant-related genes and their role in severe COVID-19: insights from an Italian cohort

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Background: COVID-19 presents variable clinical manifestations, ranging from asymptomatic to life-threatening conditions. The virus binds angiotensin-converting enzyme receptors on alveolar epithelial type-II cells, impairing the production/secretion of pulmonary surfactant. This impairment increases alveolar surface tension, causing collapse, interstitial pneumonia, and acute respiratory distress syndrome. This study investigates the genetic predispositions within surfactant-related genes that could contribute to severe COVID-19.

Material and Methods: We analysed the genetic contribution of 26 surfactant-related genes in 527 Italian patients with severe COVID-19 with respiratory failure and 3,215 controls from the general Italian population. After QC, 8,2 million single-nucleotide polymorphisms (SNPs) were retained for common and rare variant association studies using PLINK v1.9.

Results: A total of 42,869 SNPs spanning 250 kb up- and downstream of the 26 surfactant-related genes were tested for association with COVID-19 severity, adjusting for age/sex/age^2/age*sex and the first 10 principal components of ancestry. We identified 42 suggestive signals ($P < 1e-03$), the top one being at chr17:36955694:T:A in the ACACA gene ($P = 5.1e-05$). We evidenced additional 75 suggestive hits ($P < 1e-03$) using recessive, genotype, and dominant models, with the top hits for each being chr16:2037451:T:TG ($P = 1.02e-05$), chr16:2037451:T:TG ($P = 1.8e-05$), and chr11:73189476:G:A ($P = 1.06e-04$), in ABCA3 (two signals) and P2RY2 genes, respectively. Haplotype analysis of these top three genes confirmed ACACA association, identifying two significant haplotypes ($P = 2.7e-03$), located within the gene

and 15-kb downstream, in a region enriched in distal enhancer-like elements.

Conclusion: The implicated genes regulate cholesterol homeostasis and phosphatidylcholine metabolism, essential for surfactant function. Meta-analysis and RNA/exome sequencing will further clarify their role in severe COVID-19.

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P18.133.D New candidate genes for sweet and spicy food liking

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Background: Food liking (FL) are one of the main determinants of our diet, and are key to health status. Previous studies have highlighted their moderate/high heritability, but little is known about the genes involved. This study aimed to identify new genes/variants associated with FL and test their association with parameters related to behaviour and health status.

Material and Methods: Approximately 2500 Italian individuals, for whom FL, genetic/genomic data, and health information were available, were included in the study. GWAS were performed on sweet and spicy FL. Regression models were applied to verify the relationship between the most associated SNPs and health parameters.

Results: As regards sweet foods, we found two interesting associations:1) for the liking of milk chocolate, a region in which the most associated SNP ($p < 5 \times 10^{-8}$) is an eQTL for the *LARP1* gene in Nucleus Accumbens, a central driver of reward response controlling the pleasantness and gratification given by food. This SNP was interestingly associated also with impulsivity ($p < 0.05$).2) For the liking of a group of sweet foods, the *RGS9* gene, that we also found associated with reward dependence and BMI ($p < 0.05$). Regarding spicy foods, we identified an association ($p < 1 \times 10^{-7}$) near the *DOCK3* gene that is expressed in the central nervous system. The most associated SNP is an eQTL for *CACNA2D2*, a gene already associated with FL and caffeine/coffee consumption.

Conclusion: These findings represent an important starting point for identify the genes involved in FL. Further research is needed to better elucidate their role on FL and health status.

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P19 Genetic Epidemiology and Mendelian Randomisation

P19.001.B Mosaic loss of chromosome Y is a risk factor for coronary atherosclerosis in men: data from the SCAPIS study

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Background: Atherosclerosis is the most common form of cardiovascular disease and the primary cause of myocardial infarction and stroke. Men have an increased risk of atherosclerosis, which can only partly be explained by cardiovascular and environmental risk factors. We hypothesize that mosaic loss of chromosome Y (mLOY), a male-specific chromosomal aberration, contributes to the male predominance. mLOY has previously been associated with adverse cardiovascular events, but no association with atherosclerosis is reported.

Material and Methods: The Swedish CArdioPulmonary bioImage Study (SCAPIS) is a population-based cohort studying cardiovascular and pulmonary disease. Atherosclerosis imaging information and genotyping data are available for the 30,000 participants aged between 50–64 years. Genotyping data was used to estimate mLOY in the male participants using the MoChA pipeline. Multivariable logistic regression analyses were performed to assess if mLOY in blood is associated with atherosclerosis, measured by coronary computed tomography (CCTA) and carotid ultrasound.

Results: We observed an association between having >10% mLOY in blood and presence of any coronary atherosclerosis (OR = 1.42, 95% CI = 1.04–1.97, p-value = 0.0318) and substantial coronary stenosis (OR = 1.53, 95% CI = 1.03–2.21, p-value = 0.0288), measured by CCTA. No association was detected with occurrence of carotid plaques. We further show that men with >10% mLOY exhibit an increased accumulation of calcified plaques (coronary artery calcium score >= 100, OR = 1.43, 95% CI = 1.05–1.93, p-value = 0.0195), indicating a higher risk of future cardiovascular events in this group.

Conclusion: This novel correlation between mLOY and coronary atherosclerosis could explain the overrepresentation of atherosclerosis in men, plausibly driving the known association between mLOY and major cardiovascular events.

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J. Bjurling: None. **J. Halvardson:** None. **L. Forsberg:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Cray Innovation AB.

P19.002.C Integrative polygenic score and single cell transcriptomic analysis reveals differed features among healthy individuals

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Background: Polygenic scores (PGS) have been widely used for estimating individual risk to a complex trait by assessing the combined effects of multiple genetic variants identified through GWAS. We hypothesize that healthy individuals with a high PGS for a complex disease are likely to display distinct molecular signatures indicative of their predisposition to disease risk.

Material and Methods: We utilized data from the OneK1K cohort, which includes genotype and single-cell RNA-seq data of peripheral blood mononuclear cells (PBMC) from nearly 1000 individuals. Polygenic risk scores (PGS) were computed for each individual across 10 complex traits including monocyte count, leukemia, and autoimmune conditions. Individuals in the top and bottom 5th percentile of PGS for each trait were selected for comparison at the single-cell level, focusing on the cellular composition, differential gene expression, and functional pathways.

Results: Our results reveal that B cells in healthy individuals at high risk of lymphoid leukemia display heightened activity influenced by BAFF activity, whereas individuals predisposed to autoimmune diseases exhibit dysregulated genes associated with immune regulation. The presence of overlapping dysregulated

genes in individuals at high risk of systemic lupus erythematosus (SLE) and those diagnosed with SLE lends support to the notion that susceptible individuals exhibit traits related to the disease.

Conclusion: Our study integrates PGS analysis with scRNA-seq to identify characteristics in healthy individuals with high polygenic scores. Exploring the functions of these dysregulated genes in high-risk individuals may reveal key factors influencing disease susceptibility, enhancing our understanding of disease initiation and development

C.O.I. Disclosure:

Q. Wang: None. **X. Dang:** None. **W. Yang:** None.

P19.003.D Unravelling Diabetic Cardiomyopathy through genetic and epigenetic profiling

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Background: Diabetic Cardiomyopathy (DC) develops in a subset of Type-2-Diabetes (T2DM) patients characterized by cardiac dysfunction independent of ischemic diseases or hypertension. The underlying causes of DC remain unknown. This study, as part of the CARDIATEAM Consortium, aims to dissect the genetic and epigenetic landscape of DC, employing Polygenic Risk Scores (PRS) and Methylation Risk Scores (MRS) for enhanced risk stratification.

Material and Methods: We analyzed 173 deeply clinically phenotyped participants from a discovery cohort, grouped into two subsets defined by the severity of T2DM related cardiac dysfunction, with a prevalence of T2D among more severe individuals. PRS was calculated after genotype imputation with effect sizes from a T2DM Polygenic Score (PGS) [PGS001357]; MRS employed effect sizes from 145 CpGs identified in the Generation Scotland Study as strongly associated with T2DM. We evaluated four logistic regression models incorporating PRS, MRS, their combination and interaction.

Results: Both T2DM-based PRS and MRS had significantly different distribution between the groups (p-value 0.02 and 1.29e-05, respectively) with significant ORs at percentiles ≥80th and ≥90th (2.23 and 2.82 for PRS, 4.52 and 3.49 for MRS, respectively). Selecting the individuals with the highest PRS and MRS values, we obtained a significantly higher OR (6.48) for ≥80th. The combined model demonstrated the best discriminative ability (AUC = 0.753).

Conclusion: This study highlights the interplay between genetic and epigenetic factors in DC, being able to identify individuals at high risk to develop severe T2DM related cardiac dysfunctions and paving the way for improved screening and personalized management strategies.

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P19.004.A Discovery of new loci associated with albuminuria using a multi-ancestry GWAS meta-analysis

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Background: Understanding albuminuria, a key marker of chronic kidney disease, requires identifying genetic variants and genes affecting urinary albumin-to-creatinine ratio (UACR) through genome-wide association studies (GWAS). Multi-lateral collaborations and rigorous, centralized data quality control (QC) are essential for reliable results.

Material and Methods: We utilized our newly developed "metaGWASmanager" package for streamlined QC workflow. Studies performed genotype imputation using state-of-the-art reference panels such as TOPMed, including low-frequency variants. Standardized scripts were distributed to all studies for conducting phenotype harmonization and GWAS. Ancestries were validated by principal component analysis on allele frequencies. A newly developed hierarchical fixed-effects meta-analysis workflow was performed with METAL to identify global and ancestry-specific loci.

Results: We aggregated 75 GWAS on UACR encompassing 816,577 individuals across six ancestries: 61% European (EUR), 15% East Asian (EAS), 14% African (AFR), 5% Admixed American (AMR), 4% South Asian (SAS), and 1% Middle Eastern (MID). Overall, we identified 108 associated loci of which 49 are novel compared to our previous CKDGen Consortium meta-analysis. Additional MR-MEGA analysis confirmed the homogeneity of the known locus at *CUBN* (rs45551835, p_assoc = 1.9e-170, p_ancestryhet = 0.53). Ancestry-specific meta-analyses identified 91 loci in EUR, 13 in EAS, and 3 in AFR, with 10, 1, and 1 being unique to these ancestries, respectively.

Conclusion: By incorporating participants from diverse ancestries, we address the limitations of prior studies focused primarily on European ancestry. Results expand the understanding of the genetic underpinnings of albuminuria. The identified loci represent promising targets for further functional follow-up studies and potential therapeutic targets for kidney disease.

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P19.005.B Reducing Selection Bias in Biobanks: Improve Generalizability of Inverse Probability Weights by Accounting for Population Prevalences of Diseases

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Background: A recognised limitation within epidemiological studies is the non-representative nature of the sample due to selective participation. However, collecting detailed population phenotypic and genetic data is often impractical. To ensure validity in downstream analyses, it is important to employ methods that adjust for selection bias, ensuring the sample accurately reflects the broader population. Within recent literature Schoeler et al.(Nat Hum Behav 2023), use inverse probability weights (IPW) to correct for this participation bias, however their approach may not be suitable for health outcomes affecting probability of participation, e.g., psychiatric disorders and severe health outcomes.

Methods: Extending the least absolute shrinkage and selection operator (LASSO) model used by Scholer et al., we propose an additional regularization term that accounts for nationally reported population disease prevalence. We will perform downstream analyses, such as genome-wide association studies and Mendelian randomization, after correcting for selection bias through this adjusted reweighting in the Danish iPSYCH sample and UK Biobank to demonstrate differences in estimates.

Results: In simulations the IPW with an additional LASSO regularization term demonstrated a marked reduction in population-sample differences of disease prevalence, relative to the unadjusted LASSO approach. Applied analyses to demonstrate the impact of this sample correction in downstream analyses, such as genome-wide association studies and Mendelian randomization, are ongoing.

Conclusion: Our simulated results demonstrate that it is possible to reduce selection bias within large scale biobanks for both common and rare traits. Mitigation of discrepancy between sample and population distributions will improve the validity and generalisability of estimates in health and disease research.

C.O.I. Disclosure:

C.S. Barry: None. **G. Hemani:** None. **B.J. Vilhjálmsson:** F. Consultant/Advisory Board; Modest; Allelica.

P19.006.C Causal relationships between gut microbiome and age-related traits

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Background: In the past 20 years, the involvement of gut microbiome in human health has received particular attention, but its contribution to age-related diseases remains unclear.

Material and Methods: We performed a comprehensive investigation of 4,033 potential causal relationships between 37 traits representing gut microbiome composition and function and 109 age-related phenotypes, through the causal inference method two-sample Mendelian randomization (MR). We used inverse variance weighted (IVW), as main method to assess significance and weighted median and MR-PRESSO methods as sensitivity analyses. For IVW we employed a false discovery rate (FDR) correction to control for multiple testing within each outcome. Pleiotropy (egger intercept), heterogeneity (Cochran's Q statistics), leave-one-out and reverse MR were considered as sensitivity analyses. Finally, we performed replication with independent datasets to ensure strength of results, along with a post-hoc power analysis.

Results: Five causal relationships remained significant after multiple testing correction and sensitivity analysis, specifically between two taxa of *Coriobacteriales* and the risk of developing age-related macular degeneration ($p_{FDR} = 0.047$), species *Bifidobacterium adolescentis* and levels of TNFSF12 protein in plasma ($p_{FDR} = 0.0003$), and the lactose-galactose degradation microbial I pathway and levels of IL-15Rα ($p_{FDR} = 0.03$) and TRAIL ($p_{FDR} = 0.006$) proteins in plasma. The causal relationship between the microbial pathway and TRAIL protein levels was further confirmed using independent data ($p = 0.01$).

Conclusion: These results support the role of gut microbiome in regulating the inflammatory circuit and the importance of rigorous methodologies and replication to establish causality in MR studies. However, future studies are needed to investigate the underlying biological mechanisms.

C.O.I. Disclosure:

F. Grosso: None. **D. Zanetti:** None. **S. Sanna:** None.

P19.007.D Exploring causal mechanisms of sex dimorphism in lipid metabolism using the UK Biobank Pharma proteomics data

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Background: Lipid traits are known to be sex-dimorphic, but the underlying sex-specific molecular players are largely unknown. Since protein levels are downstream products of gene expression, proteomics data can be crucial to understand sex-differences in lipids metabolism.

Material and Methods: We used sex-specific pQTL summary statistics for 1,462 circulating proteins measured in the UK Biobank Pharma Proteomics Project (UKB-PPP), and sex-specific summary statistics of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) from the Global Lipids Genetics Consortium. We performed additional analyses in the Lifelines cohort. A total of 1,366 and 1,389 proteins in men and women respectively had genome-wide significant hits and thus were selected as exposures for two-sample Mendelian Randomization (MR) analyses. MR was carried using two strategies: both cis- and trans-acting variants, and cis- only variants.

Results: A total of 51 and 28 proteins showed significant causal effects exclusively in women and men, respectively (p value after false discovery rate correction). The Cochran's Q test based on betas detected a total of 20 proteins showing significant sex-different causal effects, specifically 12 in the cis-trans analysis and 15 in the cis-only analysis. Out of these, 8 showed significant effect in the reverse MR and thus were considered inconclusive. Finally, using the Lifelines cohort we rule out the possibility that those results were driven by individuals under lipid-lowering drug therapy.

Conclusions: These results highlight the need to perform sex-stratified pQTL mapping and provide insight into potential genetic drivers of sex dimorphism in lipid metabolism.

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P19.008.A Genome-wide association study meta-analysis reveals novel loci associated with polyps of the female genital tract

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Background: Female genital tract (FGT) polyps are benign overgrowths affecting women of all ages. While often linked to abnormal uterine bleeding, up to 82% are asymptomatic, leading to underdiagnosis. Estimated to be present in up to 50% of women, about 2.7% become malignant. Genetic drivers of FGT polyps and their genetic links to reproductive cancers remain understudied.

Material and Methods: We meta-analysed genome-wide association studies (GWAS) of FGT polyps from the UK Biobank, FinnGen, Estonian Biobank, and the Michigan Genomics Initiative (44,227 cases, 473,321 controls) and performed a transcriptome-wide association analysis (TWAS) to identify genes at significant GWAS loci. Mendelian randomisation (MR), and both global (LDSC) and local (LAVA) genetic correlation analyses were used to assess the genetic interplay between FGT polyps and a range of gynecological disorders.

Results: GWAS meta-analysis identified 25 FGT polyp risk loci, including a strong signal near *EEFSEC* (3q21.3), a region implicated in endometrial cancer and endometriosis risk. TWAS results highlighted 104 genes across multiple tissues, including *EEFSEC*. LDSC and LAVA revealed strong significant genetic correlations between all gynecological disorders, while MR revealed causal pathways between FGT polyps, endometrial cancer and uterine fibroids.

Conclusion: We reveal shared genetic relationships between FGT polyps and gynaecological diseases, and highlight *EEFSEC*'s potential role in conditions affecting the endometrium. Future aims include replicating findings and investigating the identified gene roles in polyp formation within endometrial cell lines.

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P19.009.B Optimization of Multi-Ancestry Polygenic Risk Score Disease Prediction Models

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Polygenic risk scores (PRS) have ushered in a new era in genetic epidemiology. However, PRS-based models still need to overcome several hurdles before they can be broadly applied in the clinic. Chiefly, they need to achieve sufficient accuracy, easy interpretability and portability across diverse populations. Leveraging trans-ancestry genome-wide association study (GWAS) meta-analysis, we generated novel, diverse summary statistics for 30 medically-related traits. We benchmarked the performance of six existing PRS algorithms using UK Biobank and built an ensemble model using logistic regression, which was validated on the diverse eMERGE and PAGE MEC cohorts. Our ensemble model surpassed current state-of-the-art PRS models, with minimal performance drops in external cohorts, indicating good calibration. Our PRS model for coronary artery disease identified 55–80 times more true coronary events than rare pathogenic variant models, reinforcing its clinical potential. Notably, the polygenic component modulated the effect of high-risk rare variants, stressing the need to consider all genetic components in clinical settings. To enhance predictive accuracy, we incorporated easily-accessible clinical characteristics creating disease prediction models intended as prospective diagnostic tests, with easily interpretable positive or negative outcomes. After adding clinical characteristics, 12 out of 30 models surpassed 80% AUC. Further, 25 traits exceeded the diagnostic odds ratio (DOR) of 5 and 19 traits exceeded DOR of 10 for all ancestry groups, indicating high predictive value. These findings show that newly developed PRS-based disease prediction models have sufficient accuracy and portability to warrant consideration of being used in the clinic.

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P19.011.D Investigating maternal and perinatal causes of breastfeeding outcomes through Mendelian randomization

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Background: Breastfeeding is a critical biological function providing essential health benefits to both mothers and offspring. Yet, in high-income countries many women stop breastfeeding shortly after birth and 44% never breastfeed exclusively. Genetic and modifiable risk factors likely contribute to this complex trait. We aimed to provide robust evidence about the causal role of maternal (socio-demographic, psychological, cardiometabolic) and perinatal factors on breastfeeding.

Material and Methods: We used individual-level data from 3 European cohorts (MoBa N = 60000, ALSPAC N = 7000, UKBB N = 4000) to conduct novel GWAS of breastfeeding traits including exclusive/initiation/establishment/duration, and meta-analyzed association results using fixed-effect inverse variance weighted approach. We performed two-sample Mendelian randomization (MR) to determine the causal effects of maternal and perinatal factors on breastfeeding outcomes. Genetic instrumental variables were extracted from publicly available GWAS. We compared MR results with those from confounder-adjusted multivariable regression (MVA) and ran extensive sensitivity analyses.

Results: MR results showed that higher maternal education causally increased the odds of initiating and sustaining breastfeeding, while smoking, obesity, high cholesterol and sleep disorder were detrimental. Specifically, a 1 kg/m^2 BMI increase corresponded to 20% lower odds of initiating breastfeeding and reduced the duration of exclusive breastfeeding by 0.5 weeks. Smoking and the diagnosis of sleep disorders reduced the odds of breastfeeding initiation by 57% and 43%, respectively. These causal associations were robust in sensitivity analyses including bias due to genetic pleiotropy and replication in MVA.

Conclusion: We provide causal evidence on maternal cardiometabolic liabilities and psychological stressors for suboptimal breastfeeding, identifying intervention targets to improve mother-offspring well-being.

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P19.012.A Integrating single-cell and population-scale data to explore immune cell type variation and disease associations

Daniela Zanotti[1,2], *Alessandro Raveane*[1], *Arianna Landini*[1], *Matiss Ozols*[3], *Stephen Watt*[3], *Shuang Li*[3,4], *Olli Dufva*[3], *Cardinal Project Working Group*[5], *Francesca Ieva*[1,2], *Sarah A. Teichmann*[3,6], *Oliver Stegle*[7,8], *Nicola Pirastu*[1], *Nicole Soranzo*[1,3,4]

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The immune system is dynamic, shaped by genetic and environmental factors. Variability in immune cell composition measured from clinical full blood counts has been linked to disease predisposition. Single-cell RNA sequencing (scRNA-seq) enhances resolution by identifying cell states. Integrating genomic data, scRNA-seq, and electronic health records informs how genetic variation and environmental factors influence cell abundance and disease risk.

We examined relationships between genetic variation, cell compositional traits, and 339 diseases using data from the CARDINAL study: 3,597 individuals from UKBiobank, 895 of Pakistani and Bangladeshi origin from Genes & Health. To overcome scRNA-seq compositional constraints, we derived absolute cell counts by interpolating proportions of 26 cell types with Sysmex full blood counts. We fitted linear models testing associations between absolute counts and disease diagnoses accrued over a 30+ year follow-up period. Then, we performed a Genome-Wide Association Study (GWAS) to identify genetic variants driving cell count differences.

Age and smoking were significantly associated with defined monocyte, T, and B cell populations. We identified 39 disease-cell-type associations, including CD14+ monocytes and naive B cells linked to obesity and type 2 diabetes. GWAS revealed 19 significant loci, some previously associated with cell type ratios, like rs3826007, linked to CD14+/CD16+ monocyte ratio. Our analysis showed that CD16+ counts drive the signal, while decreased CD14+ reflects compositional effects.

We show that population-scale single-cell studies reveal how age, lifestyle, disease, and genetics shape the immune system. The identified variants will help uncover potential causal roles of cell type abundance in disease, informing therapeutic development.

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P19.013.B Modifiable Risk Factors for Alzheimer's Disease: a Mendelian Randomization perspective

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Background: Despite extensive efforts, modifiable risk factors for Alzheimer's disease (AD) are still poorly understood. The long prodromal phase of AD, the presence of confounders, competing risk factors, and the risk of reverse causation make the detection of causal risk factors particularly challenging. To address these challenges, we used Mendelian Randomization to investigate the role of modifiable risk factors in AD.

Methods: We considered 30 genetically instrumentable risk factors as exposures. We used the AD GWAS from the IGAP consortium as the primary outcome, which ensured thorough classification and age-matching of cases and controls. Using this data, we performed extensive bidirectional univariable and multivariable Mendelian Randomization (MR) analyses.

Results: Whilst we could validate the well-known protective effect of intelligence and the risk-increasing role of LDL cholesterol, the effect of other risk factors did not align with the previous literature. Namely, we found evidence that a higher BMI reduces the risk for AD, mediated by lean mass. Furthermore, higher systolic blood pressure (SBP) robustly reduced the risk for AD. This effect was neither driven by competing risk factors nor by reverse causation, but we could replicate the risk-increasing effect of higher SBP on traits related to vascular dementia (VaD).

Conclusion: Beyond well-established risk factors for AD (such as intelligence and LDL cholesterol), we also found that higher SBP and higher lean mass had a robust protective effect on AD. Our findings demonstrate the importance of carefully assessing distinct components of exposures to understand how they relate to different forms of dementia.

C.O.I. Disclosure:

L. Knüsel: None. **I. Foote:** None. **Z. Kutalik:** None.

P19.014.C Proteomic insights into the impact of exogenous hormones on breast cancer: A two-stage observational and Mendelian randomization study

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Background: Breast cancer is the most common cancer among women worldwide. While exogenous hormones are known to increase the breast cancer risk, the underlying mechanisms remain unclear. This study aimed to identify proteins that mediate the effect of exogenous hormones on the risk of developing breast cancer.

Material and Methods: In a two-stage study, we first estimated the association with the current users of hormonal contraceptives (HC) in 5,242 premenopausal women and menopausal hormone therapy (MHT) in 16,500 postmenopausal women on the level of 2,923 plasma proteins in the UK Biobank. HCs and MHTs were categorized by hormonal content and route of administration. Second, we performed two-sample Mendelian randomization analyses to identify proteins causally associated with breast cancer.

Results: After multiple test correction, we identified seven proteins that were significantly associated both with use of any of the medications and causally linked to breast cancer development. These proteins, including ENG, EphA4, ICAM5, PTPRM, OMgp, PTPRK, and CST6, were considered potential mediators of the effect of exogenous hormones on breast cancer, with all of them having functions highly relevant to breast cancer development.

Conclusion: Our results suggest that the effect of exogenous hormones on breast cancer is partly mediated through different proteins. The results agree with that the estrogen component and predominantly oral medications have the greatest effect on protein levels. Identifying these proteins and their effects could potentially reveal new intervention targets for mitigating the effects of HC and MHT on breast cancer risk.

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P19.015.D Blood pressure as a mediator of the potential causal effect of circulating plasma proteins on coronary artery disease and stroke: A Mendelian randomisation and observational study

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Background: The biological pathways leading to elevated blood pressure (BP) and subsequent cardiovascular diseases (CVDs) remain incompletely understood. This study employed comprehensive proteome-wide Mendelian randomisation (PWMR) and observational analyses to investigate the shared proteomic landscape between BP, coronary artery disease (CAD), and stroke.

Material and Methods: A two-sample PWMR (cis-MR) study was conducted to assess the potential causal associations of 2,007 plasma proteins on BP and CVD, leveraging the largest available genome-wide association studies. The inverse-variance weighted method, or Wald ratio, was used as the primary method, complemented by robust MR approaches and Bayesian colocalization to mitigate confounding due to linkage disequilibrium. Network MR evaluated BP's mediating role in the effects of plasma proteins on CVD. Findings were further validated using the UK Biobank through comparative analysis and Cox proportional hazards models.

Results: PWMR and Bayesian colocalization analysis prioritized 13 plasma proteins potentially causally linked with BP (false discovery rate < 0.05; colocalization probability > 70%), with five proteins (ACOX1, BRAP, FGF5, FURIN, MST1) also associated with CAD and/or stroke risk. These associations were successfully replicated in the International Consortium for Blood Pressure (p-value < 0.05). Network MR revealed that BP mediated 30.4%–77.2% of the proteins' effects on CVD. These findings were supported by observational analyses, and downstream analyses showed no evidence of adverse effects from perturbing these proteins on other outcomes evaluated.

Conclusion: This study highlighted key proteomic features common to BP, CAD, and stroke, with BP potentially playing a major mediating role in their effects on CVD outcomes.

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P19.016.A Implementation of precision genomics in healthcare - NGS analysis workflow streamline

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Background: Personalized medicine tailors medical care using genetic, lifestyle, and environmental data. Advances in next-generation sequencing (NGS), including whole genome sequencing (WGS) and clinical/whole exome sequencing (CES/WES), enable genomics to transform healthcare by improving disease prevention. However, its adoption in clinical practice is hindered by the need for standardized, practical workflows.

Material and Methods: This study introduces a pipeline for variant filtering and interpretation using CES, WES, or WGS in 109 patients. The study categorized high-confidence variants into groups such as carrier status, cardiovascular disorders, hereditary cancer, and pharmacogenetics. Variants were prioritized using ACMG pathogenicity criteria and computational tools. Additional approach targeted variants of uncertain significance (VUS), prioritizing non-sense and frameshift variants in critical regions for future evaluation, ensuring clinically actionable insights. Polygenic scores for coronary heart disease, type 2 diabetes were also calculated and reported.

Results: The vast majority of patients >95% carried pathogenic variants for rare diseases, with a median of four variants. Pharmacogenetic analysis revealed poor and intermediate metabolizers in relevant pharmacogenes in 1/3 of patients, influencing drug efficacy. Actionable variants linked to arrhythmias, hereditary cancer, thrombotic risks, as well as high polygenic risk scores were identified, prompting specialist evaluations. VUS findings in genes associated with conditions such as thoracic aortic aneurysm and Lynch syndrome, required ongoing monitoring.

Conclusion: This workflow demonstrates the value of genomic profiling in clinical practice by providing actionable insights while addressing challenges like standardization and cost-effectiveness. This pipeline establishes a foundation for precision genomics in mainstream medicine.

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P19.018.C Financial stress and sleep duration in immune and neuroendocrine patterning. An analytical triangulation in ELSA

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Background. Proinflammatory and neuroendocrine mediators are implicated in disease aetiopathogenesis. Stress increases concentrations of immune-neuroendocrine biomarkers through a complex network of brain-body pathways. Suboptimal sleep further modulates these processes by altering major effector systems that sensitise the brain to stress. Given the ubiquitous, impactful nature

of material deprivation, we tested for a synergistic association of financial stress and suboptimal sleep with these molecular processes.

Material and Methods: With data drawn from the English Longitudinal Study of Ageing (ELSA), associations were tested on 4,940 participants (~66 ± 9.4 years) across four-years (2008–2012). Through analytical triangulation, we tested whether financial stress (>60% insufficient resources) and suboptimal sleep durations (≤5/≥9 hours) were independently and interactively associated with immune-neuroendocrine profiles, derived from a latent profile analysis (LPA) of C-reactive protein, fibrinogen, leukocytes, cortisol, and insulin-like growth factor-1.

Results: A three-class LPA model offered the greatest parsimony. After adjustment for genetic predisposition, sociodemographics, lifestyle, and health, financial stress was associated with short-sleep cross-sectionally (RRR = 1.45; 95%CI = 1.18–1.79; $p < 0.001$) and longitudinally (RRR = 1.31; 95%CI = 1.02–1.68; $p = 0.035$), and it increased risk of belonging to the *high-risk* inflammatory profile by 42% (95%CI = 1.12–1.80; $p = 0.004$). Suboptimal sleep was not related to future risk of *high-risk* profile membership, nor did it moderate stress-biomarker profile associations.

Conclusion: Results advance knowledge by revealing how inflammation and neuroendocrine markers cluster in older cohorts and respond to financial stress over time. Financial stress associations with short-sleep are supported. The null role of suboptimal sleep, as exposure and mediator, in profile membership, provides valuable insight into the dynamic role of sleep in immune-neuroendocrine processes.

C.O.I. Disclosure:

O.S. Hamilton: None. **A. Steptoe:** None.

P19.019.D Evaluating the effects of genetically proxied metformin`s HbA1c lowering effects on aortic stenosis and coronary artery disease: A Mendelian randomisation study

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Background: Aortic stenosis (AS) and coronary artery disease (CAD) frequently co-occur, contributing to a significant cardiovascular burden. While metformin, a widely prescribed oral antihyperglycaemic agent, has demonstrated potential protective effects on CAD, its impact on AS remains unexplored. In this work we aimed to investigate the causal effects of metformin on AS and CAD incidence in the general population.

Material and Methods: We conducted a two-sample Mendelian randomisation (MR) study using genetic instruments associated with gene expression of known metformin targets (including AMPK, MC-1, MG-3, GDF-15, GLP-1/GCC, FBP-1, and ADCY1) and decreased HbA1c. Effect sizes were obtained from UK Biobank for HbA1c, a GWAS for AS (14,819 cases and 927,044 controls), and a CAD GWAS (34,541 cases and 261,984 controls) all from European ancestry. The random-effects inverse variance weighted (IVW) method was used for the primary analysis and each of MR-Egger, simple-median, simple-mode, weighted-median and weighted-mode were applied for the sensitivity analysis. An observational analysis was also performed within UK Biobank.

Results: Our MR analysis showed a protective effect of genetically proxied HbA1c lowering effects of metformin targets against CAD (OR = 0.60, 95% CI: 0.42–0.85) but not AS (OR = 0.92, 95% CI: 0.48–1.76). These findings were consistent across various sensitivity analyses, confirming the robustness of the results. The observational analysis yielded consistent results for AS but not for CAD.

Conclusion: While metformin demonstrates a protective role in CAD warranting further investigation, its efficacy for AS appears to be limited and hence can be deprioritised for AS treatment.

C.O.I. Disclosure:

Y. Sbeih: None. **C.P. Nelson:** None. **L. Stoma:** None.

P19.020.A PolyGenie: Deciphering Genetic Liability Across Diseases in the GCAT Cohort

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Background: Pleiotropy, where a single genetic variant influences multiple traits, is pervasive in the human genome. The finite number of segregating sites and the increasing number of Genome-Wide Association Studies (GWAS) underscores the significance of pleiotropy in understanding non-communicable diseases and the interplay between genetic and environmental factors affecting disease risk. Leveraging the GCAT cohort, we developed PolyGenie, a web-based tool for exploring polygenic risk score (PRS) associations across diseases and traits.

Material and Methods: We calculated approximately 100 PRSs for a deeply phenotyped cohort of 20,000 volunteers from Catalonia, leveraging real-world data such as Electronic Health Records, metabolite levels, detailed questionnaires, and environmental exposures. Our pipeline integrates GWAS summary statistics to estimate individual genetic liability and associate it with diseases, metabolite levels, and self-reported traits. PolyGenie is a dynamic resource, with plans to continuously expand by incorporating additional PRSs to cover a broader range of diseases and traits.

Results: PolyGenie facilitates PRS phenome scans, providing visual and quantitative insights into the associations between specific PRSs and a wide range of traits and diseases. For instance, we identified associations between the genetic liability for frailty and late-onset conditions such as obesity and gastroesophageal reflux disease. Additionally, the tool allows users to explore these associations by visualizing disease prevalence across PRS percentiles, stratified by sex or analyzed collectively, offering a more nuanced understanding of disease patterns and their genetic underpinnings.

Conclusion: By uncovering shared genetic bases across diseases and phenotypes, PolyGenie enhances our understanding of genetic influences on health and disease.

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P19.021.B Joint impact of reproductive success and monogenic and polygenic risk on breast cancer

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Background: Reproductive success and timing are strongly associated with breast cancer (BC), with earlier age at first birth (AFB) and higher parity offering protective effects. Whether

reproduction modulates hereditary BC risk, conferred by single-gene pathogenic variants (PV) and polygenic risk score (PRS), remains unestablished.

Materials and methods: We addressed this question in 200,000 genotyped Finnish women (>45 years) with over 20,000 confirmed BC diagnoses and comprehensive health-registry data on reproductive history available.

Results: BC incidence increased with higher AFB (HR = 1.028/year), lower lifetime parity (HR = 0.891/delivery), and higher PRS (HR = 1.685/SD). When analyzed jointly, AFB and parity stratified BC risk across PRS categories. For example, nulliparous women in the top PRS decile were at 8.7X greater lifetime BC risk compared to women with ≥3 deliveries in the lowest decile. We additionally noted positive additive interaction between AFB>35 and high genetic risk (RERI = 3.6).

GWAS performed for AFB, parity, and BC pointed to largely independent effects of reproduction and BC genetic predisposition. Only ER-positive BC and AFB displayed genetic correlation (rg = 0.1119, P = 0.0002), with no shared BC associations identified within the 36 AFB and parity loci. E.g., the signals at the ESR1-locus were distinct between BC and AFB.

AFB and parity stratified risk also among rare BC PV carriers. For instance, in CHEK2 c.1100delC carriers (N = 3161), higher AFB increased BC risk (HR = 1.021, p = 0.0152), while parity had no detectable effect, potentially reflecting shared biologic mechanisms.

Conclusion: These findings underscore the importance of considering reproductive behavior in BC risk management particularly in individuals at elevated genetic risk.

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P19.022.C Sex-specific predictive performance of polygenic scores for type 2 diabetes

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Background: Genetic contribution to type 2 diabetes (T2D) susceptibility can be summarised in a polygenic score (PGS), offering the potential to enhance prevention strategies for individuals at high risk. However, current disease-predictive models overlook the sex-dependent effects of genetic risk factors, instead focusing on the different baseline risks for men and women. Although T2D prevalence in men and women is comparable, a prominent difference in prediabetes frequency may reflect distinct underlying biology. This study aims to systematically evaluate the sex-specific predictive performance of polygenic scores for T2D available in the Polygenic Score Catalog (PGSC).

Material and Methods: Using electronic health records of Estonian Biobank (EstBB) participants, we identified 13,818 T2D cases (63% women) and 189,634 controls (66% women). The analysis was restricted to T2D scores developed without EstBB data and published from 2019 to 2023 in the PGSC. A total of 55 PGSs were calculated for EstBB genotype data with pgsc_calc. The effects of PGSs on T2D risk were estimated using Cox proportional hazard models.

Results: We found that 50 scores had a statistically significant (FDR P-value < 0.05) hazard ratio per standard deviation of genetic risk, ranging from 1.06 to 1.56. No difference in the distribution of effect sizes in men and women was observed (P-value = 0.702, Wilcoxon test). Lastly, the analysis showed that 8 out of 10 best predictive scores for men and women are the same.

Conclusion: Taken together, these findings reveal no support for the substantial sex-specific predictive performance of publicly available T2D polygenic scores.

C.O.I. Disclosure:

V. Rukins: None. **K. Läll:** None. **T. Laisk:** None. **R. Mägi:** None.

P19.023.D Robust within family Mendelian randomisation analyses of dairy consumption using the LCT variant

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Background: The long-term effects of dairy consumption on human health are unclear as long running randomised controlled trials are impractical and traditional observational epidemiological studies are subject to latent confounding. Population based Mendelian randomisation (MR) studies have utilised a variant in the lactase persistence gene (rs4988235) to proxy dairy consumption. However, this variant is subject to strong geographical variation, and consequently such MR studies are potentially confounded by uncorrected population stratification, dynastic effects and/or assortative mating. These processes may produce spurious findings and/or conceal genuine causal relationships.

Material and Methods: We performed within family tests of association using the rs4988235 variant in 19,074 sibships from the UK Biobank study and compared our results to those from an independent population-based subset of 349,779 unrelated individuals. We investigated 38 continuous and 52 binary medically relevant outcomes.

Results: Within family tests of association were largely consistent with population-based results including significant associations between the allele for lactase persistence and decreased levels of apolipoprotein-A/HDL-cholesterol. Interestingly a subset of traits showed association with lactase persistence in the within families analysis, but not the population based analysis including decreased risk of "fractured bones in the last five years".

Conclusion: We are in the process of replicating our findings in independent sibships. Our results highlight potential problems with relying on population-based MR studies alone for causal inference and the advantages that can be obtained by leveraging within family MR.

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P19.024.A Longitudinal genomic prediction of chronic diseases in Southern Europe

Federica Santonastaso[1], *Simona Costanzo*[2], *Edoardo Giacopuzzi*[1], *Chiara Chiereghin*[1], *Davide Bolognini*[1], *Arianna Landini*[1], *Soumick Chatterjee*[1], *Alessandro Gialluisi*[2,3], *Amalia De Curtis*[2], *Emanuele Di Angelantonio*[1,4], *Giovanni de Gaetano*[2], *Maria Benedetta Donati*[2], *Nicola Pirastu*[1], *Licia Iacoviello*[2,3], *Nicole Soranzo*[1,4,5]

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Background: Non-comunicable diseases pose significant challenges to global health. Polygenic risk scores (PRSs) have emerged as powerful tools for identifying genetic susceptibility, improving risk prediction, and informing prevention strategies. This study leveraged data from the Moli-sani study, a prospective cohort of 24,325 adults (≥35 years, 49.3% men) recruited in the Molise region of Italy, to evaluate the utility of PRS in predicting disease risk in Southern Europe.

Material and Methods: PRS weights were derived from genome-wide association studies (GWAS) in European populations. Cox proportional-hazards models were used to examine the association between PRS and the risk of major diseases—including breast, prostate and colorectal cancer, and coronary heart disease (CHD)—over 15 years.

Results: PRSs were significantly associated with the 15-year risk for breast cancer (HR[95%CI] = 1.70[1.51–1.90], $p = 8.05 \times 10^{-20}$), prostate cancer (HR[95%CI] = 1.99[1.79–2.21], $p = 1.24 \times 10^{-38}$), colorectal cancer (HR[95%CI] = 1.32[1.18–1.46], $p = 3.52 \times 10^{-7}$), and CHD (HR[95%CI] = 1.40[1.31–1.49], $p = 1.07 \times 10^{-25}$). Multivariable analysis showed that PRS added predictive value beyond baseline risk factors and family history. For example, CHD-specific PRS enhanced predictive accuracy, independently of family history and Framingham score (HR[95%CI] = 1.40[1.31–1.50], $p = 2.17 \times 10^{-21}$). Furthermore, PRS showed that individuals with a greater genetic predisposition were diagnosed earlier, as expected due to lifelong higher exposure to risk. For example, women with high breast cancer PRS reached equivalent cumulative risks 10 years earlier than their low-risk counterparts.

Conclusion: PRSs effectively stratify people with different disease risk and predict long-term health outcomes in the Italian population. These findings underscore the potential of PRS in precision medicine, particularly for early detection and personalised prevention of diseases.

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P19.025.B Association of *TMEM173/STING1* gene variants with severe COVID-19 among fully vaccinated vs non-vaccinated

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Background: The cGAS-STING pathway plays an important role in the innate immune response against pathogens. There are many studies that indicate that this pathway is involved in the pathogenesis of COVID-19. We investigated the association between common variants in the STING/TMEM173 gene and the risk of developing severe COVID-19.

Material and Methods: We genotyped two STING1 gene variants (rs78233829 and rs1131769) in 906 patients that require hospitalization due to COVID-19, including 400 patients attended in the Intensive Care Unit (ICU). We obtained the main clinical and analytical values, including the vaccination status (RNA vaccine). Early onset was a <65 years. The different groups were statistically compared with R.

Results: Allele and genotype frequencies for the two *STING1* variants did not differ between severe (ICU) vs less severe cases. However, there were significant differences between vaccinated (N = 105) vs non-vaccinated (N = 801) (p = 0.003, OR = 2.13, 95%, CI = 1.29–3.50). The *LZTFL1* variant that was associated with the risk of severe COVID-19 did not differ between the two vaccination groups.

Conclusion: *STING1/TMEM173* gene variants were associated with the risk of developing severe COVID-19 among vaccinated. Our results indicate that these common variants could serve to identify individuals with a reduced vaccination response, that would be at increased risk for developing severe COVID-19

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P19.026.C ApoE Polymorphism Analysis in Health and Disease of South Asian Populations

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Background: This systematic review and meta-analysis assesses the distribution and health implications of apolipoprotein E (ApoE), which play crucial roles in lipoprotein metabolism, in South Asian populations, with a focus on neurodegenerative, movement-related, mental health, cardiovascular,metabolic and trauma-related disorders.

Material and Methods: A total of 53 studies identified through comprehensive searches in PubMed, Embase, and Google Scholar up to July 31, 2024, were included on the basis of predefined eligibility criteria after Risk Of Bias Assessment via the New York Ottawa Scale. Meta-analysis was performed using random-effects model, pooling allele frequency and genotype proportions with 95% confidence intervals. Subgroup analyses were conducted by disease type, reporting pooled logarithm of odds ratios and p-values for associations. The study was registered in PROSPERO (registration number CRD42024575197).

Results: The most prevalent APOE genotype was ε3/ε3 (73.7%), followed by ε3/ε4 (14%), ε2/ε3 (7.5%), ε2/ε4 (0.785%), ε4/ε4 (0.764%), and ε2/ε2 (0.35%).ε4-containing genotypes were found to be strongly associated with susceptibility to Alzheimer's disease, coronary artery disease, vascular dementia, and obesity, whereas the ε2/ε3 showed significant protective effects in Alzheimer's Disease.

Conclusion: This review reflects South Asian specificity of ApoE polymorphism–disease associations, highlighting the need for targeted genetic research and tailored public health strategies to advance personalized medicine and healthcare policies in this region. The review had several limitations, including data gaps for specific health conditions, underrepresentation of some South Asian countries, and heterogeneity in outcomes.

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P. Paudel: None. **A. Sah:** None. **P. Paudel:** None.

Test of association [logOR(p value)] of ApoE genotype with selected diseases														
	Alzheimer's Disease (n = 11)	Coronary Artery Disease (n = 8)	Vascular Dementia (n = 8)	Dementia with Lewy Body (n = 2)	Fronto Temporal Dementia (n = 2)	Mild Cognitive Impairment (n = 2)	Parkinson's Disease (n = 2)	Stroke (n = 3)	Diabetes Mellitus (n = 3)	Dyslipidemia (n = 2)	Metabolic syndrome (n = 2)	Obesity (n = 2)	Depression (n = 1)	Traumatic Brain Injury (n = 1)
ApoE e2/e2	0.05(0.92)	−0.47(0.1)	0.37(0.52)	0.62(0.61)	−1.00(0.56)	−0.75(0.53)	0.47(0.74)	0.07(0.88)	0.62(0.44)	−0.14(0.91)	−0.11(0.84)	−0.57(0.59)	0.68(0.74)	−1.4(0.34)
ApoE e2/e3	−0.37(0.05)	−0.26(0.14)	−0.27(0.29)	−0.23(0.65)	−0.71(0.16)	−0.71(0.59)	−0.52(0.05)	−0.28(0.15)	−0.10(0.43)	−0.06(0.97)	0.07(0.8)	0.24(0.35)	0.69(0.63)	0.36(0.33)
ApoE e2/e4	0.93(0.04)	0.34(0.26)	0.75(0.1)	1.45(0.16)	0.94(0.47)	1.39(0.24)	0.22(0.75)	1.44(<0.001)	0.25(0.45)	−1.16(0.29)	0.00(0.74)	−0.17(0.68)	0.68(0.74)	0.85(0.1)
ApoE e3/e3	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
ApoE e3/e4	1.32(<0.001)	1.10(<0.001)	0.52(<0.001)	0.78(0.43)	0.46(0.49)	0.75(<0.001)	0.98(<0.001)	0.29(0.28)	−0.12(0.23)	−0.07(0.83)	0.12(0.17)	0.09(0.55)	1.2(0.13)	−0.16(0.65)
ApoE e4/e4	1.95(<0.001)	1.29(0.09)	1.13(<0.001)	2.18(0.1)	0.34(0.76)	0.28(0.6)	0.71(0.18)	0.11(0.75)	−0.02(0.97)	1.71(0.01)	0.50(0.15)	1.47(0.01)	0.28(0.74)	−0.24(0.75)

Pooled prevalence of ApoE genotype and pooled ApoE allele frequency in general population			
Genotype	**Pooled Prevalence in % (95% CI)**	**I^2 (Heterogeneity)**	**p value (Heterogeneity)**
ApoE e2/e2	0.35 (0.20, 0.50)	15.07	0.012
ApoE e2/e3	7.5 (6.6, 8.4)	72.2	<0.001
ApoE e2/e4	0.785 (0.6, 1)	54.8	<0.001
ApoE e3/e3	73.7 (71.3, 76)	90.57	<0.001
ApoE e3/e4	14 (12.1, 15.9)	91.36	<0.001
ApoE e4/e4	0.764 (0.6, 1)	33.77	0.062
Allele	Pooled allele frequency (95% CI)	I^2 (Heterogeneity)	p value (Heterogeneity)
Apoe e2	0.0529 (0.045, 0.06)	89.58	<0.001
Apoe e3	0.851(0.838, 0.865)	92.16	<0.001
Apoe e4	0.0885(0.079, 0.098)	88.16	<0.001

P19.027.D Shared Genetic Architecture and Novel Loci in Alzheimer's Disease, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, and Parkinson's Disease: A Multi-Trait Genome-Wide Association Study

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Background: Neurodegenerative disorders such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Lewy body dementia (LBD), and Parkinson's disease (PD) exhibit distinct clinical features but often show overlapping pathophysiological processes suggesting potential shared molecular mechanisms. We aimed to study the genetic architecture of these diseases and identify novel as well as pleiotropic genetic loci.

Material and Methods: We applied Multi-Trait Analysis of GWAS (MTAG) on AD, ALS, LBD, and PD and leveraged their genetic correlations to detect novel loci. A subsequent multi-trait colocalisation analysis using brain single-cell expression quantitative trait loci (eQTL) was performed on the MTAG signals to prioritise candidate causal variants (Posterior probability > 0.7, percentage explained by variant > 75%), shared genes and their cells of expression.

Results: The MTAG analysis identified 4 novel loci for AD, 3 for ALS, 4 for LBD, and 1 for PD (Table). The colocalisation analysis highlighted 3 candidate causal variants. Specifically, rs6733839 (colocalised with AD and LBD) was an eQTL for *BIN1* in microglia; rs199529 (colocalised with ALS and PD) was an eQTL for *LRRC37A2* in microglia; and rs2526378 (colocalised with AD and ALS) was an eQTL for *RNF43* in oligodendrocyte progenitor cells.

Conclusion: Our study provides novel insights into the shared genetic basis and mechanisms of neurodegenerative diseases highlighting candidate genes that may play a pivotal role in disease pathogenesis, and potentially guiding the development of therapeutic strategies.

Novel loci for the examined neurodegenerative diseases				
rsID	**Effect/Other Alleles**	**Effect**	**P-value**	**Mapped Gene**
Alzheimer's disease				
rs145330152	C/A	0.12	2×10^{-8}	*ASH1L*
rs7766822	G/A	0.04	1×10^{-8}	*SLC2A12*
rs62073157	G/A	0.03	3×10^{-8}	*RP11-259G18.3*
rs645544	A/G	0.03	2×10^{-8}	*SLC9A8*
Amyotrophic Lateral Sclerosis				
rs34311866	C/T	0.05	4×10^{-13}	*TMEM175*
rs17573509	G/A	0.04	1×10^{-9}	*MAPT*
rs7412	T/C	0.06	2×10^{-11}	*APOE*
Lewy Body Dementia				
rs2737002	G/A	0.06	9×10^{-19}	*SNCA*
rs1532276	C/T	0.04	1×10^{-10}	*CLU*
rs4502227	G/A	0.03	7×10^{-10}	*SETD1A*
rs199531	T/C	0.05	1×10^{-16}	*NSF*
Parkinson's Disease				
rs4566208	A/G	0.05	1×10^{-8}	*NCOR1*

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F. Koskeridis: None. **C. McKechnie:** None. **P. Elliott:** None. **P. Matthews:** None. **A. Dehghan:** None. **I. Tzoulaki:** None.

P19.028.A Nature vs nurture: unraveling social and genetic architecture of nine complex traits using All of Us

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Background: Social Determinants of Health (SDoH) significantly increase chronic disease risk and disproportionately affect marginalized communities. Integrating SDoH with Polygenic Risk Scores (PRS) could improve disease prediction; however, SDoH collection is nonuniform. Therefore, we explored various methods for defining and integrating SDoH into clinical risk prediction models with PRS across nine diseases including diabetes, heart disease, and asthma.

Material and Methods: We leveraged the All of Us Program to develop five domain-specific SDoH scores (economic stability, education, neighborhood environment, social and community context, and healthcare access) and a composite score encompassing all domains via measurement models. Baseline clinical regression models, adjusted for age, sex, and EHR-specific covariates, were incrementally expanded to four different models including: PRS or SDoH alone, both (joint model), and an interaction model (PRS*SDoH). We used area under the curve (AUC) to compare the predictive performance of models using composite scores and those incorporating simpler individual-level and area-level SDoH metrics.

Results: No single SDoH domain consistently outperformed the others, with individual-level income achieving AUCs comparable to composite scores. Individual-level SDoH measures generally outperformed area-level measures. Models incorporating SDoH improved predictive accuracy for certain diseases more than others, with the most predictive SDoH measure varying by disease. Some traits and domains had significant interactions between PRS and SDoH.

Conclusion: Simple SDoH measures performed comparably to complex composite scores in health outcome models. Disease-specific variations in SDoH associations suggest distinct "social architectures," highlighting the need for tailored, disease-specific approaches in integrating SDoH and PRS to improve prediction accuracy.

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P19.029.B Investigating epigenetic changes in body mass index trajectories across childhood

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Background: Body mass index (BMI) exhibits a non-linear trajectory across childhood. The genetics and epigenetics of cross-sectional BMI have been well studied, yet little is known about the epigenetic contribution to childhood BMI trajectories. Using the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort we modelled BMI trajectories across childhood and assessed their association with DNA methylation (DNAm) at birth.

Methods: We derived 12 phenotypes that capture distinct features of non-linear BMI trajectories (age and BMI at adiposity peak and rebound; slope and area under the BMI curve (AUC) across infancy, early childhood, late childhood, and adolescence) by fitting 260,870 repeated BMI measurements (birth-18 years) in a mixed linear model. We estimated the proportion of phenotypic variance captured by all DNAm probes in our dataset (OREML analysis) and then performed epigenome-wide association studies (EWAS) of these phenotypes and three birth traits (birthweight, birth length, ponderal index) using cord blood DNAm ($n = 835$).

Results: OREML analyses suggested that cord blood DNAm in this dataset accounts for little phenotypic variance of the BMI trajectory traits. Consistent with this, there was no evidence of association with any probes in the EWAS ($P < 1.14 \times 10^{-7}$). Probes significantly associated with birth outcomes had weak associations with all BMI trajectory traits.

Conclusion: Neonatal blood DNAm profiles have strong associations with birth outcomes but are not predictive of BMI trajectory traits. Our study illustrates the unique opportunity that longitudinal cohorts provide to better understanding the epigenetic underpinnings of BMI trajectories across childhood, which can help identify critical development periods.

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P19.030.C Lifetime estimation of time-varying BMI effects on diabetes using Mendelian Randomization and Functional Data Analysis

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Background: The causal effects of some exposures can vary throughout an individual's lifetime, with specific periods playing a more significant influence on health outcomes or revealing the long-term effects of risk factors.

Material and Methods: Using data from the UK Biobank, we examined the impact of time-varying genetically predicted BMI on T2D risk through various methodologies since their relationship across different life stages remains uncertain. First, we applied Factorial Multivariable Mendelian randomization (FM-MR) to estimate the effects of childhood and adulthood BMI, while exploring potential genetic interactions between them. Second, we focused on adulthood BMI (ages 50–70), treating it as a time-varying exposure. Initially, we assumed a cumulative effect of the time-varying trajectories of BMI on T2D risk. Then, low-dimensional factors extracted from functional principal component analysis were integrated into a Multivariable MR model to assess the time-varying causal effect.

Results: MF-MR analysis revealed that early-life BMI was non-significant, while high adulthood BMI showed a significant causal effect on T2D risk. Interaction terms were not significant, leading us to focus on adulthood BMI. Analysis of cumulative effects demonstrated a strong causal relationship between time-varying BMI during adulthood and T2D risk. Notably, functional MR analysis indicated that high BMI between ages 50 to 60 had the strongest association with T2D risk.

Conclusion: This study highlights the importance of understanding how risk factors evolve over a lifetime and their varying impacts on disease risk. Identifying critical exposure periods provides insights into clinical pathways and supports targeted prevention strategies.

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P19.031.D Multi-Omics Integration for Identifying Early Molecular Signatures of Pleural Mesothelioma in a Prospective Cohort

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Background: Pleural mesothelioma (PM) is a rare, aggressive malignancy primarily caused by asbestos exposure, with poor prognosis and limited treatment options. The molecular mechanisms underlying PM remain unclear, limiting opportunities for early detection and targeted therapies. Multi-omics integration offers a powerful approach to uncovering disease-associated pathways, improving risk assessment, and advancing biomarker discovery.

Materials and Methods: Multi-Omics Factor Analysis (MOFA) was applied to integrate genomic, DNA-methylation, miRNA, proteomic, and exome data from a nested case-control study within the EPIC cohort (142 preclinical PM cases and matched controls). Variance-capturing factors across omic layers were identified. Logistic regression models, adjusted for age, sex, and asbestos-exposure, assessed associations with PM risk. Model performance was evaluated using the AUC.

Results: High-variance factors, including Factor 5, showed a significant association with PM risk (OR = 17.09, 95%CI: 8.77–38.32, $p < 3.15e\text{-}14$) and correlated with genomics data. Adding Factor 5 to a null model increased the AUC to 0.87, improving risk stratification. Functional annotation highlighted immune regulation pathways and estrogen signaling. Methylation changes implicated XBP1_01 and HOXB4 target genes, which are associated with overall survival in mesothelioma. Dysregulated miRNAs (hsa-miR-4516, hsa-miR-486-5p) highlighted pathways associated with aging, immunity, and inflammation. Proteomic and exome analyses confirmed enrichment in pathways shaping the tumor microenvironment and supporting PM progression.

Conclusion: This study identifies key molecular signatures in preclinical PM, highlighting pathways involved in immune regulation, transcriptional control, and cellular stress. These findings enhance biomarker discovery and risk assessment and provide potential targets for early detection and therapeutic intervention.

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P19.032.A Unravelling the molecular mechanisms causal to type 2 diabetes across global populations and disease-relevant tissues

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Background: Type 2 diabetes (T2D) is a prevalent disease that arises from complex molecular mechanisms. Recent large-scale efforts by the T2D Global Genomics Initiative (T2DGGI) have generated novel insights into the genetic architecture of T2D. These findings can be subsequently used to pinpoint molecular mechanisms leading to T2D.

Material and Methods: In this work, we leverage the latest T2DGGI multi-ancestry genetic associations to identify causal molecular mechanisms in an ancestry- and tissue-aware manner. Using two-sample Mendelian randomization corroborated by colocalization evidence across four global ancestries, we analyse causal effects of 20,307 genetically-predicted gene expression and 1,630 protein levels on T2D risk using blood-derived cis-quantitative trait loci (QTL).

Results: We detect causal effects of 335 genes and 46 proteins on T2D risk, with 16.4% and 50% replication in independent cohorts, respectively. Increasing diversity enables identification of more causal molecular mechanisms due to the availability of additional instrumental variables. In our study, the causal effects of five and nine genes are detected only in the African and admixed American ancestry groups, respectively, and the causal effects of six proteins are detected only in the East-Asian ancestry group. Additionally, using gene expression cis-QTL derived from seven T2D-relevant tissues, we identify causal links between 676 genetically-predicted gene expression levels and T2D risk, including novel associations such as *CPXM1*, *FAM20B* or *PTGES2*. The identified causal effects are mostly shared across ancestries, but highly heterogeneous across tissues.

Conclusion: Our findings underscore the power of tissue-informed multi-omics causal inference analyses in uncovering molecular traits driving T2D.

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P19.033.B Cell-free DNA methylation analysis reveals tissue of origin dynamics in health

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Background: Cell-free DNA (cfDNA), fragmented DNA circulating in blood plasma and other bodily fluids, serves as a minimally invasive liquid biopsy for monitoring tissue dynamics in pregnancy, transplantation, and cancer. However, knowledge gaps regarding biological variation in cfDNA—particularly related to sex, age, and intra-individual variability—impede clinical implementation. Addressing these gaps is essential to improve the accuracy and reliability of cfDNA-based diagnostics.

Material and Methods: To investigate cfDNA dynamics in health, two cohorts of healthy individuals were studied. A longitudinal cohort (n = 150; 20–88 years) was sampled at baseline and one year later, with balanced age and sex distribution. A diurnal cohort (n = 16; 20–30 years) was sampled over three days at morning, afternoon, and evening intervals. cfDNA libraries were prepared using enzymatic methyl-seq, targeting 4,989 tissue- and age-specific methylation markers, and sequenced on Illumina platforms. Cell-type deconvolution was performed using a custom algorithm.

Results: Deconvolution analysis revealed that cfDNA in healthy individuals predominantly originates from hematopoietic cells, with granulocytes, monocytes, and megakaryocytes as major contributors. cfDNA concentration and cell-type-specific turnover were significantly influenced by age, sex, BMI, and time of day. Additionally, a cfDNA methylation-based epigenetic clock demonstrated accurate chronological age prediction.

Conclusion: This study highlights the critical role of accounting for biological variables to improve the sensitivity and specificity of cfDNA-based tests. By establishing a healthy baseline, this research advances the potential of liquid biopsies for clinical applications.

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P19.035.D Transcriptomic sepsis signatures successfully stratify patients in a Thai cohort

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Background: Critical care syndromes, such as sepsis, are defined using clinical criteria that capture highly heterogeneous groups of patients. Identifying subgroups with shared biology could identify treatable traits and novel drug targets. We have previously identified transcriptomic Sepsis Response Signatures (SRS) in UK adults admitted to intensive care with sepsis (GAinS study), associated with distinct immune responses and mortality rates, but it is unknown whether these endotypes are consistent across syndromes, populations, and hospital settings. We therefore investigated SRS in the context of melioidosis, a neglected tropical disease caused by *Burkholderia pseudomallei*.

Methods: We recruited a cohort of adult patients with melioidosis (n = 371) together with matched infected (n = 158) and non-infected (n = 492) comparators from Ubon Ratchathani, Thailand, through the BurkHostGen study. We performed RNA-sequencing on whole blood samples and used our SepstratifieR algorithm to assign samples to SRS endotypes. We performed differential gene expression analysis (limma) and inferred immune cell proportions (CIBERSORTx).

Results: Melioidosis patients assigned to SRS1 had more severe infection (Kruskall-Wallis test: SOFA p = 1.1e-11; NEWS2 p = 2.5e-14) and a higher mortality rate than non-SRS1 patients (log-rank test p = 6.58e-06). We identified 4,502 genes differentially expressed between SRS1 and non-SRS1, replicating gene expression differences from the UK sepsis cohort (r = 0.73). We similarly replicated associations with inferred cell proportions, including expansion of immature neutrophils in the SRS1 endotype.

Conclusion: The SRS transcriptomic signature successfully stratified patients in a non-European bacterial infection cohort, demonstrating its portability and broader utility to identify higher risk patients with a distinct response to infection.

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P19.036.A An integrated multi-omics analysis identifies novel protein biomarkers and potential drug targets for psoriatic arthritis

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Background: Psoriatic arthritis (PsA) is a complex, chronic immune-mediated inflammatory arthropathy that currently lacks definitive biomarkers and treatment targets. Identifying novel biomarkers and treatment targets is urgently needed for effectively managing Psoriatic arthritis (PsA).

Material and Methods: We performed a comprehensive proteome-wide Mendelian randomization (MR) analysis, integrating the human plasma proteome with genome data. Candidate proteins' reliability was assessed through Protein-Protein Interaction (PPI) network analysis and validation with external datasets. Furthermore, single-cell RNA-seq analysis and functional experiments were also conducted.

Results: Proteome-wide MR analysis revealed seven plasma protein biomarkers significantly associated with PsA. Specifically, genetically predicted lower levels of NEO1 were linked to an increased PsA risk, whereas the remaining six proteins (IL23R, ERAP2, IFNLR1, KIR2DL3, CLSTN3, and POLR2F) exhibited a positive association with PsA risk. PPI analysis further supported these findings. Notably, druggability assessment revealed that scopoletin and esculetin were the two most significant drugs associated with ERAP2. Single-cell RNA-seq analysis revealed expression of IL23R, ERAP2, CLSTN3, and POLR2F in distinct T-cell subgroups of PBMCs derived from PsA patients. Furthermore, phenome-wide association studies (PheWAS) analysis assessed the potential side effects and safety as potential drug targets. Interestingly, experimental evidence showed that IFNLR1 expression is significantly upregulated under simulated inflammatory conditions.

Conclusion: This study employed proteome-wide mendelian randomization to identify seven plasma proteins associated with PsA, including IL23R, ERAP2 and IFNLR1, providing a foundation for personalized PsA treatment strategies and contributes significantly towards understanding the pathogenesis of PsA.

C.O.I. Disclosure:

X. Huang: None.

P19.037.B Evaluation of healthy lifestyle practices for obesity risk reduction in individuals with a high polygenic score

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Background: Polygenic scores (PGS) are promising tools in precision medicine; however, their application in preventive approaches remains under explored. We aimed to evaluate the impact of healthy lifestyle practices on obesity prevention in individuals with a high PGS for body mass index (BMI).

Material and Methods: The genetic risk of obesity was estimated using the BMI-PGS in four datasets from the Tohoku Medical Megabank Community-Based Cohort. One dataset (n = 9,958) was used for model development, whereas the others (n = 69,341) were used for validation. The validation datasets results were combined in a meta-analysis. Lifestyle factors were evaluated using self-administered questionnaires.

Results: Compared with the low PGS group (1st-10th percentiles), the odds ratios (OR) for obesity were 2.27 [95% CI: 2.12–2.44] in the intermediate group (11th-90th) and 4.83 [4.45–5.25] in the high PGS group (91st-100th). Obesity prevalence increased progressively, reaching approximately 48% upregulation at the highest percentile. Increased leisure-time exercise (LTE) was associated with reduced obesity risk in all groups, with an OR of 0.90 [0.87–0.94] in the high PGS group. However, high PGS and LTE individuals (top 20% of METs in the dataset) had a 3.2-fold increased risk of obesity than low PGS and minimal LTE. Similar trends were observed for other lifestyle factors, such as sodium intake.

Conclusion: Healthy lifestyle practices can reduce the risk of obesity, even in individuals with a high BMI-PGS. However, under the given study conditions, lifestyle modification alone may not fully offset for the genetic predisposition.

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P19.038.C Increasing biological understanding of causal relationships through MR extension - Investigating telomere length and aneurysm

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Background: Mendelian randomisation (MR) suggests evidence for a causal relationship between telomere length (TL) (Codd et al., 2021) and abdominal aortic aneurysm (AAA) (Roychowdhury et al., 2023). Although multiple pathways and risk factors have been implicated in AAA development, the mechanism of the TL-AAA relationship remains unknown. Blood pressure traits have particularly been highlighted as significant risk factors for AAA development.

Material and Methods: Extensions to MR such as multivariable MR (MVMR) can be used to investigate the role of multiple exposures, whilst combining univariate MR analyses in two-step MR can infer relationships. Using an instrument generated from Keaton et al., (2024), systolic blood pressure (SBP) is investigated for its potential role in the TL-AAA relationship.

Results: Early MVMR results examining the TL-AAA relationship suggest that both TL (0.528, p-value = 5.7 × 10-18) and SBP (0.007, p-value = 3.0 × 10-3) are statistically significantly associated with AAA. Consequently, two-step MR was then conducted to identify whether SBP could potentially be considered a mediator of this relationship. Separate MR analyses suggested a statistically significant relationship for the TL to SBP MR, whilst the SBP to AAA MR was inconclusive.

Conclusion: The results suggest that although TL has a causal relationship with AAA, further investigation of SBP is required to determine whether it is a mediator of this relationship, conducted

by extending two-step MR to calculate direct and indirect effects. Additionally, using network MR methods can further investigate the role of SBP, alongside other traits such as DBP, in understanding the TL-AAA relationship and its biological mechanisms.

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P19.039.D Prevalence of familial pheochromocytoma and paraganglioma syndromes - a large multicenter study

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Background: Pheochromocytoma and paraganglioma (PPGLs) have a strong genetic background, with approximately 30% of the cases caused by a germline pathogenic variant (PV).

Aim: To study the prevalence and characteristics of hereditary PPGLs in a large multicenter cohort.

Methods: A retrospective study of patients with a personal history of PPGL. All patients underwent germline DNA sequencing (whole exome or panel sequencing). Demographic, clinical, biochemical, and genetic data were gathered.

Results: Ninety patients underwent genetic evaluation (median age at diagnosis 47 [7-73]), and 29 (33.7%) had a family history of PPGL. 41 patients had pheochromocytoma, 45 had paraganglioma, and four had both. 34 patients (37.8%) carried a PV in a PPGL-related gene; *SHDB* (14 patients, 41.1%), *SDHD* ($n = 9$, 25.7%), *VHL* ($n = 6$, 17.6%), *NF1* ($n = 2$, 5.9%), *SDHC*, *SDHAF2*, and *RET* (one patient each, 2.9%). Age at diagnosis was significantly younger in the PV-carrier group vs. non-carriers (31.63 ± 2.71 vs. 50.48 ± 2.41, $p < 0.00001$). Seventeen patients with a PV had elevated levels of catecholamines, and all had purely elevated normetanephrine (NMN) levels. NMN-only secretion was associated with younger age at diagnosis vs. other secretion profiles (36.12 ± 3.69 vs. 48.85 ± 3.13 years, $p = 0.0006$). Twelve patients (13.3%) had aggressive tumor behavior, with a significantly higher rate of PV compared with patients with the non-aggressive disease ($n = 8$ [66.67%] vs. $n = 10$ [27.03%], respectively, $p = 0.013$).

Conclusion: Patients with PV in a PPGL-related gene were younger at diagnosis and had a higher rate of aggressive disease. These results underscore the importance of genetic evaluation in patients with PPGL.

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P19.040.A Assessing Idiopathic Pulmonary Fibrosis Genetic Risk Variants Beyond European Populations

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Background: Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterised by progressive scarring, respiratory decline and high mortality. Genetic studies have predominantly focused on European populations, limiting insights into the genetic landscape in diverse ancestries.

Material and Methods: We evaluated 35 previously reported IPF risk variants in clinically curated non-European IPF cases from United States and United Kingdom, alongside ancestry- and sex-matched controls (1:10 ratio) from UK Biobank. Ancestry was inferred using a random forest classifier trained on 1000 Genomes Project reference data. Association testing, adjusted for ancestry-specific principal components, was conducted separately for each population. Polygenic risk scores (PRS) based on the 35 IPF variants with weights from European studies were constructed to assess generalisability of previous IPF genetic studies across populations.

Results: We analysed 41 African, 60 South-Asian, 20 East-Asian, and 90 American ancestry IPF cases. Of the 35 variants studied, five were monomorphic in East-Asians. The *MUC5B* promoter variant - strongest IPF risk factor in Europeans, showed similar variant effects across all the ancestry groups despite differing allele frequencies and rarity in Africans and East Asians. While PRS based on these variants were significantly associated to IPF risk in non-European populations, removing *MUC5B* negated this

association in Africans and South Asians but had minimal impact in East Asians.

Conclusion: These findings highlight the need for more powered studies to understand the genetics of IPF in non-European populations. Population ancestry differences may have an impact on the generalisability and utility of reported IPF genetic risk variants.

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P19.041.B Liver fat accumulation contributes to discordant genetic risk between type 2 diabetes and coronary artery disease

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Background: Type 2 diabetes (T2D) and coronary artery disease (CAD) are complex diseases that frequently co-occur. Recent clustering analyses of T2D genetic variants identified molecular pathways associated with disease. Here, we extend to a multi-disease approach and present a comprehensive analysis of the shared genetic and molecular determinants between T2D and CAD.

Methods: Shared genetic loci for T2D and CAD were identified using PLACO on data from multi-ancestry GWAS and confirmed using colocalisation. To understand common pathways of shared variants, we clustered variants using bNMF and assessed the associations of biomarkers and disease outcomes with clusters in 378,772 UK Biobank individuals. Finally, we examined the effect of liver fat on the risk of T2D and CAD using Mendelian randomisation (MR).

Results: We identified 154 genetic loci (90 novel) shared between T2D and CAD. There were 73 with concordant effects on T2D and CAD risk, 57 with discordant effects and 24 with mixed effects. Clustering shared loci using intermediate traits identified 7 clusters with distinct circulating metabolites and associations with different biomarkers and diseases. Some of which (e.g. beta cell and lipodystrophy) were similar to previous T2D clusters. Interestingly, clusters with high proportion of discordant variants were primarily related to liver fat, impacting circulating lipids. MR analysis further suggested that liver fat and the discordant effect on T2D and CAD may be causal.

Conclusion: Discordant risk loci in T2D and CAD centre on pathways regulating liver fat and circulating lipids, providing further insights into the complex relationship between T2D and CAD.

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P19.043.D Utilising offspring genotype by proxy Mendelian randomization to investigate potential causal effects of offspring traits on their parents' health

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Background: Offspring traits can have profound effects on parental health. This is especially evident during the perinatal period, when the fetus influences maternal physiology. The causal effect of such offspring traits on their parents' health outcomes can be investigated using Mendelian randomisation (MR), using offspring genetic variants to proxy offspring phenotypes. However, as maternal and offspring genotypes are correlated, analyses should be adjusted for maternal genotype to avoid pleiotropy through the maternal genome. This is difficult to perform at scale due to the paucity of genotyped mother-offspring pairs.

Material and Methods: We introduce offspring genotype by proxy MR, that can be performed in the absence of offspring genotype by utilising spousal information (i.e., father's genotype). Because fathers transmit half of their alleles to their offspring, it follows that paternal genotype will proxy offspring genotype, which in turn will proxy offspring phenotype. As an example, we applied this method to investigate the causal effect of fetal growth (using offspring birthweight) on maternal cardiovascular health in 45,546 spousal pairs in the UK Biobank. Complementary gene-by-environment interaction (GxE) MR analyses using maternal genotype were also performed.

Results: The offspring genotype by proxy MR design found no strong evidence for a causal effect, although confidence intervals were wide. Complementary GxE MR analyses suggested offspring birthweight was unlikely to causally affect maternal cardiometabolic risk factors.

Conclusion: Our study serves as a blueprint for future investigations into the putative causal effects of offspring on their parents using MR in the absence of offspring genotype information.

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P19.044.A Polygenic prediction of estimated glomerular filtration rate in Africans

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Background: Polygenic risk score (PRS) prediction for African populations is hindered by the limited availability of genome-wide association study (GWAS) data. Although PRS derived from African American GWAS data have shown utility in other traits, their applicability in kidney function in Africans remains largely unexplored. Machine learning (ML) methods, such as PRSmix, have shown promise in improving PRS prediction in other ancestries.

Methods: We evaluated different PRS approaches for eGFR prediction in East Africans using data from the Uganda Genome Resource (UGR). Three PRS computation methods were compared: ML-based PRS integration (PRSmix), clumping and thresholding (PRSice-2), and Bayesian approaches (PRScs). PRSmix integrated 96 eGFR-specific scores using Elastic Net, applying them to the UGR dataset (n = 6,407). PRSice-2 and PRScs used the Million Veteran Program (MVP) dataset (n = 57,336) as the discovery set and UGR as the target set, with further partitioning into training (20%) and validation (80%) subsets.

Results: The results demonstrated a marked improvement in eGFR prediction with the PRSmix approach. PRSice-2 explained 0.22% of the variance in eGFR ($R^2 = 0.22\%$, $p = 0.0001634$), while PRScs explained 0.42% ($R^2 = 0.42\%$, $p = 0.000251$). PRSmix achieved a 5-fold increase in predictive power, explaining 1.16% of the variance ($R^2 = 1.16\%$). Additionally, PRSmix improved prediction for blood urea nitrogen (BUN) and cystatin-C, explaining 1.04% and 0.17% of the variance, respectively.

Conclusion: The PRSmix approach significantly enhanced eGFR prediction. Hence, further refinements are necessary to increase the proportion of variance explained and optimize clinical applications.

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P19.045.B Partitioning of the polygenic risk score for body mass index reveals eight biological mechanisms

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Background: Obesity is a major global health issue arising from a combination of environmental factors and genetic predisposition. The latest genome-wide association study (GWAS) has identified 941 susceptibility variants for body mass index (BMI). However, translating variants to genes and biological pathways remains a major challenge. Here, we clustered BMI GWAS variants based on variant-trait associations and determined their potential biological relevance to obesity.

Methods: We applied hierarchical clustering of 941 BMI variants (GIANT meta-analysis) and variant-trait associations using the GWAS catalog. To evaluate biological relevance, we constructed weighted, partitioned polygenic risk scores (PRS) per cluster and associated each z-scaled PRS with body composition, body fat, energy intake and components of metabolic syndrome in the Rotterdam Study (N = 3941). All associations were adjusted for age and sex.

Results: We identified eight clusters containing variant-trait associations indicative of body fat, inflammation, blood pressure, lean mass, metabolic syndrome, childhood BMI, insomnia and educational attainment. The body fat and inflammation partitions were associated to all body composition and body fat measurements, with the highest effect sizes for BMI (body fat: $\beta = 0.565$, $p = 1.6e\text{-}17$, inflammation: $\beta = 0.414$, $p = 3.0e\text{-}10$) and body fat percentage (body fat: $\beta = 0.689$, $p = 8.3e\text{-}12$, inflammation: $\beta = 0.466$, $p = 3.1e\text{-}6$). Furthermore, the blood pressure partition was the only partition significantly associated with systolic ($\beta = 1.145$, $p = 5.0e\text{-}4$) and diastolic ($\beta = 0.714$, $p = 7.5e\text{-}5$) blood pressure.

Conclusion: Our study suggests that variant-trait associations can partition the BMI PRS into biologically relevant mechanisms. Greater understanding of these mechanisms could help to identity novel molecular pathways regulating metabolism, thereby improving prevention and treatment of obesity.

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P. Vlaming: None. **J.G. van Rooij:** None. **J.B. van Meurs:** None. **J.A. Visser:** None.

P19.046.C Plasma protein biomarkers of type 1 diabetes (T1D) and genetic evidence for their causality

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Background: Using plasma proteomic data in UK Biobank, we aimed to identify proteins cross-sectionally associated with type 1 diabetes (T1D) and then assess genetic evidence for a causal role in T1D.

Material and Methods: T1D was defined as prescription of insulin at recruitment to UK Biobank with a record of T1D diagnosis before age 50, or self-reported diabetes before age 50 with insulin therapy within one year of diagnosis. Logistic regression was used to test associations between measured plasma protein levels and T1D in 54219 participants. To assess causality of proteins, we (i)constructed genome-wide aggregated *trans*-effects (GATE) scores and tested their associations with T1D among the remaining 366663 participants, and (ii)examined if the protein-coding genes harbour rare variants causing autoimmune diabetes or are near known T1D GWAS hits.

Results: Association with T1D in 190 cases and 36870 non-diabetic controls identified 465 proteins (Bonferroni-corrected p-value<0.001/2919). Top hits included B7 family proteins (*CD276, NCR3LG1, PDCD1LG2*) and 12 exocrine pancreatic proteins. Genetic evidence for causality was available for 61 of these 465 proteins. Thirty-seven proteins, including *CD276, NCR3LG1*, and *PDCD1LG2*, were supported by associations of T1D with the GATE score for the protein or its receptor (p-value < 0.001). Twenty-eight proteins had been reported as T1D GWAS hits, and 3 (*CD55, IL2RA, PDCD1*) are known to cause monogenic autoimmune diabetes.

Conclusion: These findings highlight the B7 family of immune checkpoint regulators in T1D pathogenesis and emphasize the importance of genetic evidence in assessment of causality of protein associations.

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P19.047.D Menopause changes the game: differential breast cancer risk in women living with obesity

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Background: Previous research suggest that obesity is associated with no or lower risk of breast cancer in young women, while older women living with obesity show an increased risk, at some point after menopause. To what extent there is a differential effect and at what age obesity becomes a lifetime risk factor, remains inconclusive. We used genetics of body mass index (BMI) to determine the effect of obesity on breast cancer across the life-course.

Material and Methods: Aalen's additive hazard model was deployed to estimate the effect of a polygenic score of BMI on incident breast cancer during life-course, at unprecedented temporal resolution. The corresponding effect of BMI was estimated using the Mendelian randomization framework, where variations in effect of the score on BMI across age was properly accounted for. Data on age, BMI and incident breast cancer were taken from UK Biobank.

Results: We identified an increasingly protective effect of obesity in young women, up to age 50 years, after which the effect begins to diminish in strength. Around 65 years of age, obesity becomes a lifetime risk factor for breast cancer. Stratification by age-at-menopause revealed a time shift in the effect on breast cancer risk of 8 ± 4 years between women with late and early menopause, which closely matched the difference in mean age-at-menopause between the two groups.

Conclusion: Our results demonstrate the existence of a differential effect of obesity on breast cancer risk throughout life linked to menopause, which likely is due to menopausal changes in hormonal metabolism.

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T. Karlsson: None. **F. Hadizadeh:** None. **M. Rask-Andersen:** None. **Å. Johansson:** None.

P19.048.A Brain-wide pleiotropy investigation of alcohol drinking and tobacco smoking behaviors

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Background: Previous studies advanced our understanding of brain mechanisms underlying alcohol drinking and tobacco smoking identifying pleiotropic links with brain structure and function

Material and Methods: To explore these pleiotropic mechanisms, we integrated data generated by the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN; up to 805,431 participants) with information related to 3,935 brain imaging-derived phenotypes (IDPs) available from UK Biobank (N = 33,224)

Results: We observed global genetic correlations between smoking behaviors and white matter hyperintensities, the morphology of the superior longitudinal fasciculus, and the mean thickness of the pole-occipital region. The latter IDP showed a local genetic correlation with the age of regular smoking initiation (hg38 chr2:35,895,678-36,640,246: rho = 1, $p = 1.01 \times 10^{-5}$). Pole-occipital region had been previously linked to smoking initiation, educational attainment, chronotype, and cortical thickness. Our genetically informed causal inference analysis using both latent causal variable approach and Mendelian randomization, linked prefrontal and premotor cortex activity and that of superior and inferior precentral sulci and cingulate sulci to drinks per week (genetic causality proportion, gcp = 0.38, $p = 8.9 \times 10\text{-}4$, rho = -0.18 ± 0.07; inverse variance weighting, IVW beta = -0.04, 95%CI = -0.07 - -0.01). This relationship could be related to the role of these brain regions in the modulation of reward-seeking motivation and the processing of social cues

Conclusion: Overall, our brain-wide investigation highlighted that different pleiotropic mechanisms likely contribute to the relationship of brain structure and function with alcohol drinking and tobacco smoking, suggesting decision-making activities and chemosensory processing as modulators of propensity towards alcohol and tobacco consumption

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P19.049.B Characterising effects of autoimmune condition liability on adverse pregnancy outcomes using Mendelian Randomization

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Background: Autoimmune conditions are often more common in women than men, diagnosed in early adulthood, and associated with adverse pregnancy outcomes. However, whether these conditions or medications used to manage them cause adverse pregnancy outcomes is unknown.

Material and Methods: We used two-sample Mendelian randomization to explore the effect of liability to ten autoimmune conditions on 15 pre-selected adverse pregnancy outcomes. Genetic instruments were selected from published GWAS and outcome genetic association data included up to 700,000 women within the MR-PREG collaboration. Main analyses used the inverse variance weighted (IVW) method. Sensitivity analyses included MR-Egger, weighted-median, excluding the HLA region, and accounting for fetal effects.

Results: We found strong evidence that liability to type 1 diabetes (T1D) increased risks of several adverse pregnancy outcomes, such as neonatal intensive care unit admission (OR = 1.03, 95% CI:1.01–1.04 per doubling in liability) and hypertensive disorders of pregnancy (OR = 1.02, 95% CI:1.01–1.02). Liability to rheumatoid arthritis also caused increased risks across outcomes, for instance on preterm birth (OR = 1.02, 95% CI:1.01–1.04). Multiple sclerosis liability had several protective effects, for example on hypertensive disorders of pregnancy OR = 0.98, 95% CI:0.97–0.99).

We also identified potential adverse effects of Hashimoto's thyroiditis, ankylosing spondylitis, systemic lupus erythymatosus, and systemic sclerosis. For coeliac disease, inflammatory bowel disease and psoriasis, there was limited evidence supporting any effects, though several estimates were imprecise. Sensitivity analyses largely supported main IVW results and suggested that the HLA region drove some effects.

Conclusion: Our preliminary findings suggest effects vary between autoimmune conditions.

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P19.050.D Leveraging plasma proteomics and genomics to prioritise determinants of*MYBPC3*-related hypertrophic cardiomyopathy severity and potential pleiotropic effects on related cardiac diseases

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van Tintelen[2,4], Karin Y. van Spaendonck-Zwarts[2,4,5], A. Floriaan Schmidt[1,3,6]

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Background: Hypertrophic cardiomyopathy (HCM) is a highly variable disorder often caused by pathogenic *MYBPC3* variants. It may present with asymmetric left ventricular (LV) hypertrophy, LV outflow tract obstruction (LVOTO), or heart failure (HF).

Material and Methods: We studied 268 Olink-assayed plasma proteins in 144 *MYBPC3* carriers classified as unaffected, mildly, or severely affected (LV maximum wall thickness (LVMWT) ≥20 mm, LVOTO necessitating septal reduction therapy, HF, or malignant ventricular arrhythmia). Using a multi-tiered approach, we applied random forests and Firth regression to identify proteins associated with HCM severity, followed by replication and annotation based on tissue expression, druggability, and pathway analysis. Independent replication was obtained in 50,000 UK Biobank participants, including 100 HCM patients, and through Mendelian randomisation of HCM, HF, LVOTO, and LVMWT.

Results: Twenty-seven proteins associated with HCM severity in 52 unaffected (75% female; LVMWT 10±1 mm), 17 mildly affected (59% female; LVMWT 15 ± 2 mm), and 75 severely affected (31% female; LVMWT 19 ± 5 mm) *MYBPC3* carriers, of which 17 were independently replicated. NT-proBNP, MB, FGF-23, C1QTNF1, and NCAM1 are mainly cardiac proteins, while ADM, CXCL16, and CCL16 are highly expressed in secretory tissues, such as the liver. ADM is targeted by enibarcimab, a drug in development for HF. Five other proteins are targeted by drugs in development, and 11 by approved drugs. CXCL16 and CCL16 play a role in Gαi signalling, contributing to hypertrophy and contractile dysfunction.

Conclusion: This study identified and prioritised 8 proteins associated with HCM severity in *MYBPC3* carriers, providing biomarkers and therapeutic targets for HCM.

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P19.051.C Preventive genomics and clinical surveillance. A prospective and multicentric experience

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Background: Recent technological highlights such as whole genome sequencing (WGS) and bioinformatics make it feasible to plan prospective genetic screenings in healthy population. In the last years, some studies have suggested advantages in disclosing actionable information regarding genetic risk of several diseases, reducing mortality and morbidity after close surveillance.

Material and Methods: A 10,000 individual prospective project using WGS was designed in our hospital group. Age of inclusion ranged from 30 to 65 years. DNA was extracted using Qiasymphony and Illumina DNA PCR-Free WGS was performed using Illumina Xplus machines (~30x depth genomes were accepted). GRCh37 reference assembly was used. A 293 actionable gene panel was designed (CLINVAR, NCCN, ESMO, AHA, ACMG, others). Pathogenic and likely pathogenic variants were disclosed only. Genetic counseling was provided to all patients. In case of high-risk result, clinical health plans were prepared for surveillance.

Results: 2648 patients were sequenced till end of January 2025 (1712 women). 454 patients (17%) disclosed actionable variants, being HFE the main detected variants (282 patients). Approximately ~5% of our patients (140) showed linkage to a more severe condition, including, cancer risk (72 patients), heart disease risks (31 patients) or pancreatitis susceptibility (23 patients). This project is still going, and further clinical data will be disclosed at the end of it.

Conclusion: Sequencing actionable gene panels in general population discloses presymptomatic patients at risk of disease (17%, 5% of them linked to severe conditions). This approach would help healthcare systems in prioritizing and surveilling such patients from general population.

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P19.052.A Summary Statistic-Derived Metabolite Ratio QTL Analysis Identifies 427 Otherwise Missed Loci and Reveals Catalyzing Enzymes

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Background: Metabolic pathways are dysregulated in diseases like type 2 diabetes. Yet, quantitative trait locus (QTL) studies focus on one biomarker at a time. Metabolite ratios are established proxies for pathway activity, however, their use in QTL studies has been limited due to the quadratic computational burden of pairwise combinations.

Materials and Methods / Results: We developed a statistical framework to compute ratio QTL (rQTL) from existing single metabolite QTLs (mQTL) summary statistics. Validating on 299 classically derived ratios (from individual-level data), we find strong concordance (β: $R^2 = 0.87$). After application to all (261,003) pairwise ratios of 723 metabolites we identified 17,454 rQTL with stronger significance than either constituent metabolite at Bonferroni

significance ($P < 2 \times 10^{-13}$), of which 437 rQTLs were not study-wide significant ($P < 5 \times 10^{-11}$) in the original mQTL study. The closest genes to the novel rQTLs were enriched for lipid metabolism (Reactome: $P = 4 \times 10^{-14}$). *Cis* Mendelian randomization and colocalization analysis identifies 9 proteins significantly influencing 521 metabolite ratios (MR-link-2 $P < 1 \times 10^{-6}$, coloc PP4 > 0.9). All proteins were enzymes, including sulfotransferase 2A1 (SULT2A1) protein abundance as a causal regulator of the ratio between dehydroepiandrosterone sulfate and etiocholanolone glucuronide (novel rQTL $P = 2 \times 10^{-15}$): two testosterone metabolites.

Conclusion: Our framework enables scalable and resource-efficient rQTL mapping from summary statistics, bypassing the need for individual-level data. We identify previously unknown metabolite pathway regulation, efficiently resolving rQTLs, otherwise understudied as phenotypes for QTL mapping.

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P19.053.B Toward equitable chemotherapy : Insights from pre-chemotherapy DPYD genotyping implementation in Finland

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Background: Fluoropyrimidines are widely used in colorectal cancer (CRC) treatment, but 10–30% of patients experience severe toxicity (Grade 3–4), partly due to deficient dihydropyrimidine dehydrogenase (DPD) enzyme activity. Pre-treatment *DPYD* genotyping mitigates this risk by enabling personalized dosing. Using the World Health Organization's (WHO) Health Impact Assessment (HIA) guidance, we evaluated the feasibility, equity, and impact of systematically implementing *DPYD* genotyping in Finland through policy-driven integration.

Material and Methods: A structured HIA framework was applied, incorporating stakeholder engagement, oncologist and laboratory surveys, patient interviews, and Markov modeling. Quantitative analysis assessed health outcomes, while qualitative inputs captured operational challenges and patient acceptability.

Results: *DPYD* genotyping is widely adopted in Finnish oncology practice, with a reported prescription rate of 94.7%. Markov modeling showed that expanding from current adoption (94.7%) to full implementation (100%) led to marginal reductions in severe toxicity (23.9% to 23.6%) and toxicity-related mortality (0.12% to 0.11%). Challenges include test result delays (≥10 days in some regions), infrastructure disparities, and laboratory inefficiencies. Patient interviews revealed low awareness of *DPYD* testing but strong willingness to accept it, provided delays are minimized. Stakeholders assumed equity in access to be high, consistent with Finland's universal healthcare system.

Conclusion: Integrating DPYD genotyping systematically enhances patient safety and optimizes resource use. Addressing laboratory capacity and enhancing patient and provider education are critical for equitable and sustainable implementation. These findings provide actionable insights for scaling personalized medicine policies in other healthcare settings.

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P19.054.C Identification of HIV restriction factors through mRNA expression variation in HIV-infected individuals

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Background/Objectives: The interaction between HIV-1 and the host immune system plays a crucial role in the natural control and progression of the infection. Previous studies have identified HIV-1 restriction factors, which are counteracted by viral "helper" proteins. The blood expression levels of these proteins vary among individuals, suggesting interindividual differences in the spontaneous control of HIV infection might lead to the identification of novel HIV restriction factors.

Methods: Study enrolled 150 participants from the Swiss HIV Cohort Study, with provided genotypes, pre-antiretroviral treatment PBMC aliquots, and viral load measurements. Using BRB sequencing, we derived gene expression profiles and assessed their associations with viral load. Mendelian randomization studies were performed on correlated genes to infer causal relationships within these associations.

Results: We employed a generalized linear model to assess the association between 792 genes and HIV viral load. Pathway analysis revealed upregulation in innate immune response and proteasome complex with increased viral load, alongside heightened mitochondrial activity and cell-cycle. Conversely, reductions were observed in ribosomal transcripts and downregulation of genes involved in cytokine-cytokine receptor interaction, including IL4R, IL7R, and TCF. Utilizing Transcriptome-Wide Mendelian Randomization, we rediscovered TRABD2A and identified new candidates as restriction factors.

Conclusion: We performed a large transcriptomic analysis of PBMCs from PLWH without antiretroviral treatment at the time of sample collection. Comparing gene expression levels and HIV-1 VL, we identified anticorrelated genes with potential anti-HIV activity non or partially counteracted by viral proteins.

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P19.055.D Application of Mendelian randomization to appraise causality of the relationship between the gut microbiome and cancer

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Background: The gut microbiome has been implicated in cancer aetiology; however, its causal relevance is not clear. Mendelian

randomization (MR) is increasingly being applied to assess the causal role of the gut microbiome on several health outcomes; however, the complexities and limitations of the method are not fully addressed in much of the current literature. Here, we aimed to appropriately apply MR to understand the role of the gut microbiome in cancer aetiology, whilst demonstrating the range of sensitivity analyses required to provide clarity in causal estimates.

Methods: Two-sample MR was used to examine the causal relationship between microbial traits from two large microbiome genome-wide association studies (mGWASs) and risk for cancers of the lung, breast, colorectum, pancreas, prostate, ovary, endometrium and oesophagus from the largest cancer GWASs, with replication sought in UK Biobank and FinnGen. A range of sensitivity analyses were performed to appraise the robustness of findings to violations of MR assumptions.

Results: Whilst our MR analyses suggested a putative causal role of 16 microbial traits across the eight cancers, few results were replicated in both UK Biobank and FinnGen and sensitivity analyses implied violations of MR assumptions, suggesting that these findings were unlikely to reflect causality.

Conclusion: Through testing for violations of core MR assumptions, our work demonstrates the importance of performing sensitivity analyses when assessing the robustness of MR findings, especially in the context of understanding the causal relevance of the gut microbiome on health outcomes.

C.O.I. Disclosure:

A.C. Dawes: None.

P19.056.A Inflammatory Signatures in Hashimoto's Thyroiditis: A Proteomic Approach to Disease Progression and Treatment Effects

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Background: Hashimoto's thyroiditis (HT) is a chronic autoimmune disorder that leads to thyroid destruction and hypothyroidism. We aimed to investigate changes in inflammatory proteins in HT patients stratified into those that are in euthyroid state (EUTHY) and those in hypothyroid state (HYPO). Additionally, we explored the impact of levothyroxine (LT4) therapy on inflammatory markers.

Material and Methods: We measured 92 inflammatory proteins using Olink Target 96 Inflammation Panel in 440 individuals (168 EUTHY patients, 99 HYPO and 173 controls). Olink_anova analysis, adjusted for age, gender, and BMI, was performed to detect proteins differentially expressed in two groups of HT cases compared to controls. P-values per assay were adjusted using the Benjamini-Hochberg method, and post hoc comparisons were performed using Olink_anova_posthoc. Comparison between euthyroid patients on LT4 therapy and euthyroid patients without therapy was also performed.

Results: The most significant results came from post hoc analyses between HYPO and control group which showed upregulation of 18 and downregulation of 4 proteins. The most significantly upregulated proteins in HYPO were IL-17C, CCL19, and CCL20 whereas the most significantly downregulated proteins were ST1A1 and CASP-8. Notably, HT patients treated with LT4 showed no significant differences in inflammatory profiles compared to euthyroid patients without treatment.

Conclusion: This is the first systematic analysis of inflammatory proteins to explore changes of immune response in HT, especially during the transition from euthyroidism to hypothyroidism. We identified several markers potentially linked to disease progression. Additionally, our findings suggest that LT4 therapy has beneficial effects on inflammation in HT.

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P19.057.B Clinical variability of the *CFTR* variant p.Arg117Cys uncovered by newborn screening for cystic fibrosis. Does the genetic background matter?

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Background: The second most common CF-causing variant identified in the Norwegian newborn screening (NBS) is the p.Arg117Cys, prior to NBS only observed in 5% of clinically diagnosed CF-patients.

Material and Methods: From 2012 to 2015, 500 samples/year were tested using Luminex-CFTR-71-variant-assay. After lowering the IRT cut-off value, 2500 samples/year were sequenced using MiSeq139-variant-assay-v1 (2015-Feb2021) and CFTR-Clinical Sequencing-v2 (Mar2021-1Feb2025). All samples with at least one allele p.Arg117Cys detected during the 13 years of screening were selected. Samples carrying p.Arg117Cys from 2012–2015 were retrieved from the NBS biobank and re-tested with MiSeqDx-CF-Clinical-Sequencing. Clinical follow-up data of the infants with screening positive results were available.

Results: Among ~30.000 samples sequenced, 47 were NBS positive carrying at least one p.Arg117Cys allele, 11 of them homozygous. There were 411 heterozygous carriers, defined as NBS negative. The frequency of p.Arg117Cys compared to other pathogenic *CFTR* variants was 18–28%: a high allele prevalence in the Norwegian population. A variable clinical spectrum was uncovered among the NBS positives, even with identical pathogenic variant on the other allele. Co-occurring previously unevaluated *CFTR* variants were observed in the whole-gene sequenced samples.

Conclusion: The knowledge of the landscape of *CFTR* variants increased with more comprehensive sequencing. Extended allele background can be of importance and include disease-modifying variants in *CFTR*. Future studies may explore how to predict the clinical variability of *CFTR* variants by combining whole *CFTR* sequencing and filtered WGS and using long-reads technologies looking for cis- and trans-regulatory factors in *CFTR* and modifying variants in *MBL2, SFTPD*, and other genes.

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P19.058.C Two-sample Mendelian randomization analyses on GWAS summary data: comparisons of instruments selection and causal effect estimation strategies

Giulia Pontali[1], *Solène Cadiou*[1], *Alessia Mapelli*[1,2], *Dariush Ghasemi-Semeskandeh*[1], *Brian Ferolito*[3], *Michele Filosi*[4], *Michela Massi*[1], *Eva*

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Background: Mendelian randomization (MR) estimates causal effects using genetic variants as instrumental variables (IVs). Single-SNP MR may overlook important genetic effects when an exposure has multiple independent IVs, potentially reducing power. Multi-SNP MR addresses this limitation, but its advantages remain unclear. This study systematically compares the performance of single-SNP and multi-SNP MR strategies using protein quantitative trait loci (pQTL) summary statistics and confirmed protein-trait causal associations.

Material and Methods: We analyzed cis-pQTL associations for 7143 proteins, from a meta-analysis of INTERVAL[1] (n = 9657) and Cooperative Health Research in South Tyrol (CHRIS)[2] studies (n = 4225). The IV selection followed three distinct strategies: (1) selecting the most significant SNP per locus, (2) identifying conditionally independent SNPs with unconditional effect sizes and errors (Unconditional), and (3) using conditionally independent SNPs with conditioned effect sizes and errors (Conditional). MR analyses were performed on 2011 phenotypes using meta-analysis data from the Million Veteran Program, UK Biobank, and FinnGen. Performance was assessed against known causal protein-trait associations (approved drug target and indication pairs) and compared using Fisher's exact tests and odds ratios.

Results: No significant difference was observed in true positives and false negative rates across the three approaches. The Unconditional multi-SNP approach identified more associations than the Conditional approach, but all methods detected a similar number of true causal associations.

Conclusion: Multi-SNP MR may capture additional signals but does not significantly improve MR accuracy. Further analyses are needed to refine instrument selection strategies and enhance causal inference.

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P19.059.D Exploring modifying genes in NF1: unraveling the genetic basis of plexiform neurofibromas and optic pathway gliomas for personalized medicine

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Background: Neurofibromatosis type 1 (NF1) is a common autosomal disorder affecting ~1 in 3,000 individuals, caused by loss-of-function mutations in the NF1 gene on chromosome 17q11.2. NF1 exhibits near-complete penetrance and involves diverse clinical manifestations, including café-au-lait macules, neurofibromas, gliomas, skeletal abnormalities, and learning difficulties. Clinical variability is significant, with age-dependent onset and unpredictable progression. While genotype-phenotype correlations are limited, modifying genes, somatic mutations, and epigenetic factors likely contribute to this variability. Modifying genes, though not causative, can alter target genes and influence phenotypic expression, making their identification crucial for understanding NF1 pathogenesis and improving personalized care.

Material and Methods: This study focuses on two frequent and clinically significant NF1 manifestations: plexiform neurofibromas (PNFs) and optic pathway gliomas (OPGs). We compare the genetic backgrounds of NF1 patients with and without these manifestations using high-resolution genomic analyses. Family-based association tests are conducted to identify potential modifying genes and recurrent genetic changes.

Results: The preliminary results are promising. The completed analysis of the first batch of data will be presented.

Conclusion: This study highlights the role of modifying genes in NF1 phenotypic variability, particularly for PNFs and OPGs. Identifying these genetic influences could improve prognostic accuracy, guide targeted therapies, and advance personalized medicine for NF1 patients. This study seeks to unravel the molecular mechanisms underlying NF1's clinical variability, offering new insights into this complex disorder.

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Q. Hao: None. **M.J. Larsen:** None. **N. Illum:** None. **L.B. Ousager:** None.

P19.060.A Identifying predictors of weight loss from a diverse set of biological, behavioural, and psychological factors

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Background: Identifying predictors of successful weight loss could guide intervention selection and resource allocation. Our purpose was to explore predictors of weight loss from 172 polygenic scores (PGS) proxying a diverse set of variables including physiological markers, medical issues and procedures, health behaviours (including dietary intake and physical activity), and personality traits.

Material and Methods: We predicted the percent of weight lost, calculated as the person's minimum weight following their highest recorded weight, in an Estonian Biobank subsample (N = 47,217) of non-weight-stable people with BMI ≥ 25 kg/m2 at baseline (i.e., at their maximum weight), with at least one follow-up weight measurement available between one month and four years from baseline. A PGS was calculated for 172 variables using SBayesR, based on summary statistics from Genome-wide association studies in the UK Biobank cohort. All predictors and the outcome were first residualized for sex, baseline BMI, 20 principal components of ancestry, and the linear and quadratic terms of age and time from baseline and minimum recorded BMI, and standardized.

Results: Greater weight loss was most strongly predicted by the PGSs for apolipoprotein B, lymphocyte percentage, neutrophil percentage, HDL cholesterol, higher education, and number of non-cancer illnesses (β = .01 ⋯ .04). Lower weight loss was predicted by the PGSs for current and past smoking, higher age at first sexual intercourse, LDL cholesterol, and pain experienced in the last month (β = .01 ⋯ .03).

Conclusion: Biological and lifestyle variables emerged as the strongest predictors of weight loss.

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P19.061.B CNV landscape of Hong Kong Chinese population and detection of rare CNV aggregation in twin pairs with specific learning disorders

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Background: Additive genetic factors of genome-wide single nucleotide polymorphisms cannot fully explain the heritability of reading and mathematical ability estimated by the twin ACE model. We investigated the characteristics of copy number variants (CNV) and rare CNVs in twin pairs with specific learning disorders to understand their role and to address the missing heritability.

Material and Methods: Low-pass whole genome sequencing was performed on 844 Hong Kong Chinese twins for CNV calling at 10-kb resolution. Rare CNVs with allele frequency <1% in 15 twin pairs with dyslexia or dyscalculia were investigated.

Results: Overall, 4099 deletions and 1872 duplications were detected in 844 twins. The minimum, median, and maximum CNV sizes were 10 kb, 48 kb, and 5.26 Mb, respectively. Over 50% of CNVs were between 10–50 kb in size, which have previously been unappreciated. 45.9% and 44.5% of deletions and duplications overlapped with at least 1 gene. Rare CNVs previously reported in patients with neurodevelopmental diseases were detected in 4/15 twin pairs with specific learning disorders, including: intronic duplications of *NEGR1* (chr1:72592570_72630203dup), partial deletions of *NDUFAF2* (chr5:60244295_60277541del), intronic deletions of *FGF12* (chr1:151332079_151406433dup), and partial duplications of *POGZ* (chr3:g.191935518_191990864dup). These genes were related to dyslexia, attention deficit/hyperactivity disorder, developmental and epileptic encephalopathy, and White-Sutton syndrome, respectively.

Conclusion: We showed the first CNV map of the Chinese general population. Aggregation of rare CNVs was observed in 4/15 twin pairs with specific learning disorders and patients with neurodevelopmental diseases. This provides preliminary evidence that CNVs account for missing heritability of academic ability.

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P19.062.C Saliva as a diagnostic medium: DNA methylation biomarkers for disorders beyond the oral cavity

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Background: Saliva is an accessible biological fluid that could potentially contain diagnostic markers for complex diseases. Methylation and expression quantitative trait loci (mQTL and eQTL) can reveal the link between GWAS SNPs and complex traits. This study explores whether genetic predisposition to common diseases is reflected on DNA methylation (DNAm) and gene expression profiles in saliva.

Material and Methods: In saliva samples from the general population, we constructed a cis-mQTL database (n = 345) to capture the impact of 5,052,978 genetic variants on DNAm levels (736,680 CpGs) and a cis-eQTL database (n = 277) with 38,040 genes. Results were integrated with the largest available GWAS on several cancers, cardiometabolic traits, and neurodegenerative disorders using Summary-based Mendelian Randomization (SMR). Additionally, we analyzed the influence of the CpG sites associated with those disorders on gene expression (eQTMs) in 271 individuals of the cohort.

Results: We created the largest public saliva-based cis-mQTL and cis-eQTL databases, identifying 123,451 mSites and 1,717 eGenes, respectively. Integration with GWAS revealed 436 CpG sites and 22 genes as potential biomarkers. Five eQTMs were identified from those CpG sites and genes pleiotropically associated with diseases, including breast and prostate cancers, squamous cell carcinoma, and coronary artery disease.

Conclusion: Our study offers a valuable resource with the largest saliva-based mQTL and eQTL databases, to facilitate biomarker discovery for systemic diseases. These findings emphasize the potential of saliva in the diagnosis of complex traits beyond the oral cavity.

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P19.063.D Charting the landscape of therapeutic and potential adverse events associated with JAK inhibition using Mendelian randomization

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Background: The JAK-STAT pathway has emerged as an effective therapeutic target for immune-mediated diseases (IMIDs). However, concerns about its positioning and potential safety risks remain. Mendelian randomization, which leverages genetic data to assess causal relationships, can be geared to characterize the safety and efficacy profiles of JAK inhibitors (JAKi).

Material and Methods: We selected 78 genes from the JAK-STAT pathway and used eQTL and pQTL variants as instrumental variables to investigate the effects of altering these genes on

IMIDs, cardiovascular risk, cancer-related malignancies, and opportunistic infections as potential adverse events.

Results: We performed a comprehensive MR analysis to evaluate the therapeutic potential and unintended effects of JAKi. Our findings confirm known associations with IMIDs, such as inflammatory bowel disease and psoriasis, supporting the relevance of the approach. The analyses also suggest potential benefits, such as JAK2 targeting associated with a reduced risk of myeloproliferative neoplasms. However, our results highlight the need for caution in specific cases where JAKi use is linked to an increased risk of adverse effects, such as herpes zoster infection.

Conclusion: Our pathway-wide analysis provides a comprehensive map of the therapeutic potential and adverse effects, while proposing novel associations that need to be pursued through epidemiological analyses and devoted trials. This approach offers a valuable framework to provide a preliminary guide to evaluate drug targets.

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P19.064.A Sex-Specific HLA Alleles Contribute to the Modulation of COVID-19 Severity

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Background: Coronavirus Disease 2019 (COVID-19) exhibits a spectrum of clinical manifestations, from asymptomatic to severe pulmonary dysfunction or death. The variability in COVID-19 severity has largely been attributed to the host's genetic characteristics, suggesting a polygenic genetic architecture, without significant evidence of sex-related genetic differences.

Material and Methods: In this Italian retrospective case-control study, we investigated the association between COVID-19 severity (severe vs. asymptomatic/oligosymptomatic healed individuals) and HLA gene variants, analyzed by next-generation sequencing (NGS).

Results: We identified significant HLA alleles (according to conventional nomenclature), SNPs and haplotypes in the HLA-B, -C, -F, -DQA1, -DRB1, and -DRB5 genes associated with COVID-19 severity. Interestingly, these variants showed biological sex-related effects. Also, we identified specific haplotypes associated with COVID-19 severity shared by different conventional HLA alleles, indicated here as "super-haplotypes". These haplotypes had a biological sex-specific impact on disease severity and markedly increased the risk of severe COVID-19 compared to conventional HLA alleles (odds ratio up to 15).

Conclusion: Our data suggest that the revision of the current HLA nomenclature may help identify variants with a stronger effect on disease susceptibility and that association studies could benefit from the stratification of patients by biological sex. If replicated in other disease models, these findings could help define the functional diversity in immune response between sexes, also based on the HLA system. Finally, due to the pandemic's mortality rate, we hypothesize here that COVID-19 may have acted as a natural selection trigger, leading to a drift in HLA allelic frequencies in the general population.

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P19.066.C Gene-environment interaction in human traits and diseases: a story of misconception

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Gene-environment interaction typically refers to generative models where the effect of a genetic variation on an outcome depends on the value of a non-genetic risk factor. In a statistical model, an interaction is defined as the product of two variables (here $G \times E$). By construction, this interaction term is correlated with the original variables, producing collinearity. For example, if E is a binary exposure with a frequency of 0.8 and G is a SNP with a minor allele frequency of 0.2, the expected correlation $cor(G, G \times E) = 0.85$. Such high collinearity can severely impact the interpretability of linear models. In the context of GxE, this has two consequences. First, estimates from interaction models are expected to have larger variance than estimates from marginal models, which explains the low statistical power of existing $G \times E$ interaction tests. Second, collinearity makes the assessment of each term's contribution to the outcome's variance challenging. The current standard, built for mathematically convenient, consists in comparing the R-squared between the marginal ($Y \sim G+E$) and interaction models ($Y \sim G+E+G \times E$), attributing most of the variance explained to the simpler model (marginal effects) and assigning what remains to more-complex terms (the interaction effect). We show that this approach systematically underestimates the contribution of GxE and poorly qualifies the expected change in genetic effect across the exposure spectrum. We propose an alternative metric, named the Pratt Index, that resolves this bias. When applied in real UK Biobank data, we measured variance explained by GxE up to five fold larger than suggested by the standard approach.

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P19.068.A The etiologic role of trimethylamine N-oxide (TMAO) in cardiometabolic diseases in East Asians: A Mendelian randomization study

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Background: Trimethylamine N-oxide (TMAO) is a novel risk factor of cardiometabolic diseases although its etiologic role remains unclear. Previous Mendelian randomization (MR) studies, a design less vulnerable to confounding, showed no association with cardiometabolic diseases albeit the instruments used were not very strongly related to TMAO. Furthermore, these studies were conducted in Europeans and hence limits generalizability to other populations, such as East Asians.

Material and Methods: We conducted a genome wide association study (GWAS) (N = 2458) to identify genetic predictors of urinary TMAO (SD of log) in a Chinese birth cohort ("Children of 1997"). Information from these instruments were applied to summary statistics from East Asian GWAS of 12 cardiometabolic diseases (N≤433540) and 11 risk factors (N≤216058) using a 2 sample MR design. Association of genetically predicted TMAO with outcomes was estimated using inverse variance weighting, followed by several sensitivity analyses.

Results: At the significance level of 5×10^{-6}, the GWAS identified 28 instruments related to TMAO. The F statistics ranged from 20.98 to 35.51, indicating low level of weak instrument bias. Genetically predicted TMAO was not associated with all cardiometabolic outcomes (FDR adjusted p value>0.571). We also observed no strong evidence for an association with cardiometabolic risk factors. Sensitivity analyses, and the use of different instrument selection criteria based on p values gave similar conclusion.

Conclusion: This study showed TMAO unlikely relate to cardiometabolic disease and its risk factors in East Asians. These null findings require further replications when larger TMAO GWAS in East Asians become available.

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P19.069.B *HLA* genotype combinations impact allele association with Multiple Sclerosis risk

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Associations between Multiple sclerosis (MS) and *HLA* involve 7 predisposing and 6 protective alleles (*HLA-DRB1*03:01/*08:01/*13:03/*15:01, HLA-DQB1*03:02, HLA-DPB1*03:01, LTA-H51P* and *HLA-A*02:01, HLA-B*38:01/*44:02/*55:01, HLA-DQA1*01:01, HLA-DQB1*03:01*, respectively). Here, we explore the effects of *HLA*-wide genotype combinations on MS associations.

We analysed WTCCC *HLA* data for 11,376 MS-cases (2005 diagnosis criteria) and 18,872 controls. We performed principal component analysis for European ancestry selection. *HLA* alleles were imputed using the *HIBAG R* package or inferred with proxy SNPs (*rs2229092, rs9273912* and *rs9277565*), and then recoded to consider only the 13 MS-associated alleles. We divided the dataset, using 20% to search for MS-associated genotype combinations and 80% to test them for replication. Replicated combinations were assessed on the whole dataset.

We retained 9,024 MS and 13,923 controls from European Ancestry. In the 20% sub-sample, we observed 776 genotype-combinations, 41 of which were nominally MS-associated ($P < 0.05$). In the 80% sub-sample, 22 of those were replicated (*P corrected* <0.05/41). In the full dataset the 14 predisposing combinations (OR 1.83–6.75) accounted for 23.61% of MS-cases and the 8 protective combinations (OR 0.30–0.57) for 4.29%. Surprisingly, some predisposing combinations carried "protective" alleles and vice versa: the MS-association of a given *HLA*-allele depends on the combination of *HLA*-wide-genotypes involving that allele. Strikingly, 4.29% of patients, diagnosed with MS prior to 2005, carried protective combinations: it will be interesting to investigate those combinations in patients who would now be diagnosed with Neuromyelitis optica spectrum disorder or Myelin oligodendrocyte glycoprotein antibody-associated disease. Those combinations could help clarifying disease heterogeneity and improve diagnosis.

C.O.I. Disclosure:

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P19.070.C Investigating the repurposing potential of cardiometabolic and hormonal drugs for cancer treatment using Mendelian randomization

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Background: Approved cardiometabolic and hormonal medications targeting hallmarks of cancer represent potential repurposing opportunities for cancer treatment. We aimed to investigate the repurposing potential of approved anti-hypertensive, lipid-lowering, anti-adiposity and anti-oestrogenic drugs for breast and ovarian cancer treatment.

Material and Methods: We obtained or constructed genetic instruments to proxy cardiometabolic traits (body mass index (BMI), low-density lipoprotein cholesterol (LDL-c), systolic blood pressure (SBP)), and anti-hypertensive (angiotensin-converting enzyme (ACE), beta-1 adrenergic receptor (ADRB1), sodium-chloride symporter (NCC)), lipid-lowering (3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), Niemann-Pick C1-Like 1 (NPC1L1), proprotein convertase subtilisin/kexin type 9 (PCSK9)), anti-adiposity (gastric inhibitory polypeptide receptor (GIPR)) or hormonal (cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1)) medication targets. The genetically proxied effects of these traits or targets on breast and ovarian cancer mortality was investigated using drug-target Mendelian randomization (MR). Current indications for these medications were employed as positive control outcomes. Assessment of potential collider bias is ongoing.

Results: Evidence was observed for genetically proxied HMGCR inhibition (HR per SD decrease LDL-c: 1.81 [1.15–2.83]) and BMI lowering (HR per SD decrease BMI: 0.89 [0.82–0.97]) on risk of breast cancer mortality, and for genetically proxied PCSK9 inhibition on risk of ovarian cancer mortality (HR per SD decrease LDL-c: 1.52 [1.06–2.18]).

Conclusion: Aside from a protective effect of lower BMI on breast cancer survival, no strong evidence was observed to support repurposing of anti-hypertensive, lipid-lowering, anti-adiposity or anti-oestrogenic drugs for breast or ovarian cancer treatment. General limitations of applying MR to cancer prognosis outcomes and collider bias may have affected analyses.

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P19.071.D Polygenic Risk scores in prediction of overall COVID-19 non-recovery

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Background: Predicting those at highest risk of ongoing symptoms after COVID-19 remains challenging. We hypothesised that incorporating genetic risk for organ impairment might improve prediction of those at risk of non-recovery, and be informative about the relationship between pre-existing conditions and longer-term recovery.

Material and Methods: Using 450 cases and 389 controls from the post-hospitalisation COVID-19 study (PHOSP-COVID) and summary statistics from large scale GWAS, we use PRSice-2 to calculate genome wide genetic risk for traits related to COVID-19 - through shared pathophysiology, similar clinical features, and previously published associations - and determine their association with non-recovery post COVID-19. We then test the hypothesis that using these genetic scores improves performance when added to a clinical prediction model of non-recovery at 12 months.

Results: Genetic risk of several traits - including BMI ($p = 0.0002$), chest pain ($p = 0.0035$), hypertension ($p = 0.0054$), T2DM ($p = 0.0057$), atrial fibrillation ($p = 0.006$), CFS ($p = 0.0111$), T1DM (0.0114), bronchitis ($p = 0.014$), IBD ($p = 0.0352$), FVC ($p = 0.0374$), anxiety/depression ($p = 0.0452$) - were nominally associated with non-recovery at 12 months. The model with clinical predictors only (AUC: 0.696, CI: 0.661–0.731) was improved by the inclusion of genetic risk of anxiety/depression alongside the first 10 genetic principal components (AUC: 0.714, CI: 0.680–0.749, $p = 0.018$), increasing total prediction accuracy from 65% to 66.5%. Other traits conferred smaller performance increases.

Conclusion: This work suggests that whilst there is some genetic overlap between COVID non-recovery and a number of traits, inclusion of genetic risk into a clinical prediction model does not meaningfully add to clinical prediction

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P19.072.A Carrier screening in the Old Order Amish and Mennonite populations in Ontario, Canada

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Background: Old Order Mennonite (OOA) and Old Order Amish (OOA) communities in Canada immigrated from Europe in the 18th and 19th centuries. They maintain a separate lifestyle with minimal influx of new genes. Founder effect has led to an extremely high prevalence of autosomal recessive disorders. Families decline Canadian universal healthcare, pay directly for medical care, and decline prenatal diagnosis. Children with treatable genetic disorders were being diagnosed late, with increased morbidity and mortality. We have sought to provide carrier screening to enhance healthcare delivery.

Material and Methods: Since 2011, targeted variant carrier screening for genetic disorders has been offered by midwives to OOA/OOM couples at their prenatal visit or when characteristic antenatal ultrasound anomalies are found. The current screen consists of 26 disorders, 12 of which have specific treatments and only 4 of which are on the provincial newborn screening panel. Babies at 25% risk have cord blood tested with 48 hour turnaround time.

Results: Carrier frequencies range from 1:4 to 1: 40, with 1:17 OOM couples and 1:7 Amish couples at risk to have an affected baby. About 6% of OOA newborns have been diagnosed at birth with one of the disorders. Rapid diagnosis of treatable disorders has resulted in timely, effective treatment for disorders such as maple syrup urine disease (BCKDHA) or biotinidase (BTD) deficiency. Families have avoided the high cost and delays of the diagnostic odyssey, especially for dual diagnoses.

Conclusion: We have demonstrated the benefits of culturally acceptable carrier screening in these genetically isolated populations.

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V.M. Siu: None. **W. McCaul:** None. **C.A. Rupar:** None.

P19.073.B The landscape of autosomal recessive pathogenic variants identified by the Carrier test in the population of Western Romania

Miruna Gug[1,2,3], *Tudor Popoiu*[4], *Nicoleta Andreescu*[2,5], *Dorina Stoicanescu*[2], *Simona Farcas*[2], *Alexandra Mihailescu*[2], *Ioana Mozos*[1,6], *Paula Roman*[7], *Cristina Gug*[2,3]

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Background: Carrier screening is used to identify individuals or couples at risk of having a child with an autozomal-recesive (AR) genetic disorder.

Material and Methods: A cross-sectional study was conducted from January 1, 2020 to December 30, 2024. The study group included 604 unaffected, unrelated individuals, of European origin, who underwent NGS using 2 multi-gene panels (164 and 302). In all cases with positive test results, a genetic consultation was performed to discuss the risk of recessive diseases in their descendants.

Results: The carrier frequency was 1.45. 469 (77.64%) individuals carried at least one AR gene mutation (range 1–7). Hemochromatosis was identified as the most common chronic condition (approx. 1 in 5). The second most common AR disorder was cystic fibrosis (1 in 9), followed by biotinidase deficiency (1:17), primary deafness related GJB2 (1:18), congenital adrenal hyperplasia caused by 21-hydroxylase deficiency (1:19), galactosemia (1:20) and Wilson disease (1:38). Our data suggest a higher risk for some AR diseases in this Romanian population, notably those associated with HFE (2 variants), CFTR (22 variants), BTD (2 variants), GJB2 (7 variants), CYP21A2 (12 variants), GALT (7 variants), PAH (12 variants), SERPINA1 (5 variants), SMN1 (3 variants) and ATP7B (8 variants) gene.

Conclusion: This is the first study in Romania that investigated the number and distribution of recessive alleles in the population of western Romania to assess the risk for various single-gene diseases. Identification of carriers of these variants must be followed by genetic counseling to inform them about reproductive risks and reproductive options.

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P19.074.C Association of genetic susceptibility to influenza infection in type 2 diabetes risk and glycemic traits: A Mendelian randomization study

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Background: Previous studies suggested influenza infection associated with increased type 2 diabetes (T2D) risk although these findings may be vulnerable to confounding. We conducted a Mendelian randomization (MR) study to explore the association of genetic susceptibility to influenza in T2D risk and glycemic traits.

Materials and Methods: We identified two single nucleotide polymorphisms (SNPs), rs16861415 (*ST6GAL1*) and rs2837112 (*B3GALT5*), strongly (p value $< 5 \times 10^{-8}$) and independently (r^2<0.001) associated with influenza infection (defined based on self reports and electronic medical records) based on a genome wide association study (GWAS) of influenza infection (Cases: 40,356; Controls: 1,407,560). These SNPs were applied to analyze the genetic susceptibility to influenza in relation to T2D and glycemic traits using corresponding GWAS (Cases: 242,283, controls: 1,569,734 for T2D, and N< = 209,605 for glycemic traits). Given the limited number of instruments, we only used inverse variance weighted method and explored pleiotropy through single SNP analyses and literature review.

Results: Weak instrument bias was unlikely based on F statistics (> 10). Genetic susceptibility to influenza infection did not increase T2D risk (OR: 1.12 per log odds, 95%CI: 0.84 to 1.49) and glycemic traits. Single SNP analyses showed a strong association of rs16861415 with T2D risk (p-value: 1.85×10^{-7}). Literature review indicated *ST6GAL1* was implicated in immune and inflammatory pathway and may be a source of horizontal pleiotropy.

Conclusion: Genetic susceptibility to influenza infection was unrelated to T2D. However, larger studies are necessary to replicate these findings, which help inform whether influenza control reduces T2D burden.

C.O.I. Disclosure:

S. Au Yeung: None. **K. Kwok:** None.

P19.076.A The contribution of the proteome to respiratory and rheumatic co-morbidities

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Background: Diseases often cluster together, but the underlying causes are not always clear. Shared risk factors, such as high BMI or smoking, may contribute, but biological mechanisms remain underexplored. Proteins play a key role in disease processes, and genetic variation can influence protein levels. This study aims to identify proteins contributing to disease co-occurrence, potentially informing drug discovery.

Material and Methods: We analysed genetic data on ~5,000 circulating proteins, assessing their role in disease pairs. This study prioritises co-morbidities between respiratory and rheumatic diseases, evaluating genome-wide genetic correlations and applying partial genetic correlation to isolate protein-driven effects.

Results: Our genome-wide analysis identified several proteins with genetic correlations across disease pairs. Notably, COPD and gout shared 106 genetically correlated proteins ($P < 0.001$), with 14 maintaining significance at $P < 0.00001$. Similarly, asthma and gout shared 47 proteins at $P < 0.001$, with 6 at $P < 0.00001$, highlighting potential biological connections. Furthermore, partial genetic correlation analysis revealed proteins with significant contributions to disease relationships. For example, *NCAN* attenuated the genetic correlation between COPD and gout from 0.185 (SE = 0.028) to 0.128 (SE = 0.030), with a t-test result of 4.36 ($P = 1.3 \times 10^{-5}$). Similarly, *SLITRK3* attenuated the genetic correlation between asthma and gout from 0.128 (SE = 0.024) to 0.079 (SE = 0.026), with a t-test result of 4.00 ($P = 6.2 \times 10^{-5}$).

Conclusion: These findings highlight proteins with potential roles in multimorbidity, offering candidates for biomarker research and drug repurposing. Future work will assess protein levels in individuals with and without disease pairs.

C.O.I. Disclosure:

R.M. Woodward: None. **F. Dudbridge:** None.

P19.077.B Romania: Remarkable Progress in Genomics Research and Personalised Medicine

Maria Puiu[1], *Octavian Bucur*[1], *Simona Olimpia Dima*[2], *Adrian Nicolae Buzatu*[1], *Simona Ruță*[3], *Stefan Busnatu*[4], *Viorel Jinga*[5]

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Background: The Institute for Research and Development in Genomics (ICDG), established by the Romanian Government and coordinated by the Carol Davila University of Medicine and Pharmacy of Bucharest (UMFCD), entered the European genomic landscape in 2023, the same year that Romania joined the "1 + Million Genomes" (1+MG) Initiative. The ICDG oversees research in genomics, bioinformatics and other Omics sciences, participates in the development of pilot projects and collaborates with 20 institutions from the National Genomics Research Network, led by UMFCD.

Materials and Methods: A key project is the Development of Genomic Research in Romania (ROGEN) and the establishment of the national reference genome, an essential resource for exploring local genetic diversity and for improving personalized diagnosis and treatment. By collecting and analysing genetic data representative of the Romanian population, this project supports precision medicine and innovation in healthcare. The ICDG also collaborates with European consortia, including the Genome Data Infrastructure, the Genome of Europe, Beyond 1 Million Genomes Plus, to foster the sustainable advancement of genomic research across Europe.

Results: Although results are pending, ICDG is already contributing to the European genomic research ecosystem by promoting the creation of the Genome EDIC and promoting the objectives of the GDI and GoE.

Conclusion: By creating a modern infrastructure, developing the National Genomic Medicine Strategy and training the workforce through European funds, ICDG, with the support of UMFCD, aim to incorporate genomics into the Romanian medical system and integrate Romanian genomics into the European context, supporting medical research and personalised medicine.

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P19.078.C Genetic Variation Underpins the Role of Hyperuricemia in Mesoamerican Nephropathy

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Background: The present study aims to characterize the genetic predisposition for the development of Mesoamerican Nephropathy (MeN) in patients from Panama.

Material and Methods: We followed a cohort of 61 MeN patients, 30 exposed healthy donors to heat stress and agricultural work and 30 non-exposed healthy donors from Central Panama for more than six years diagnosed with a comprehensive clinical, laboratory, and genetics protocol. A Whole Exome Sequencing approach was performed in patients diagnosed with MeN using the criteria of the Pan American Health Organisation (PAHO).

Results: We found a variant harbored in the APOE gene (rs429358 c.388T>C p.(Cys130Arg) belonging to the APOE4 haplotype, identified in 26% (16/61) of the patients, that exhibits an extraordinary and significant distortion of segregation (Hardy Weinberg Equilibrium of $P = 0.001$) when compared to unaffected populations from disparate regions around the world that was not found in the control groups. Blood uric acid levels in MeN patients carrying the APOE4 haplotype had a significantly higher concentration than non-carriers individuals.

Conclusion: These results may be key findings, as previous studies by our group and others suggested that uric acid metabolic dysfunction after exercise and heat stress associated with dehydration-induced hyperuricemia is frequently observed in MeN patients. We hypothesize that agricultural work at high temperatures elevates uric acid, even in healthy individuals, and that those with genetic predisposition, carriers of the APOE variant reported in this study, would be at increased risk of developing MeN.

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P19.079.D Epigenetic clocks predict cardiovascular events in a cohort of Pacific Peoples living in NZ but genetic variation may lead to spurious risk estimates

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Background: Epigenetic clocks show associations with cardiovascular disease (CVD) in non-Pacific populations. We hypothesised that epigenetic clocks may predict CVD in Pacific Peoples living in New Zealand (NZ), but clocks trained in predominantly European populations may be confounded by single nucleotide polymorphisms (SNPs) more frequent in Pacific populations.

Materials and methods: Whole-blood DNA methylation was measured with Illumina MethylationEPIC arrays in the Pasifika Heart Study community cohort ($n = 183$, Table 1). We calculated five epigenetic clocks: Horvath, Hannum, PhenoAge, Dunedin Pace of Ageing measured from the Epigenome (DunedinPACE) and Dunedin Pace of Ageing Methylation (DunedinPoAm). We tested if epigenetic clocks improve CVD risk prediction using Cox models adjusted for the linear predictor of the NZ 5-year CVD risk score (which includes 13 risk factors). Loci from each clock were screened for trimodal distributions indicative of underlying SNPs. Genotyping was confirmed with Taqman assays.

Results: The DunedinPACE, Hannum age-adjusted and PhenoAge age-adjusted clocks predicted incident CVD independently of baseline CVD risk (Table 2). The clocks constituent loci appeared free from overlapping SNPs, except for cg05513157 in DunedinPoAM, where each rs76801084 minor allele (C>T, cohort minor allele frequency = 0.18) was associated with a 0.93-fold decrease in DunedinPoAm ($p = 5.6 \times 10^{-11}$).

Conclusion: Epigenetic clocks can potentially improve CVD risk prediction for Pacific Peoples living in NZ. However, confounding SNPs present a major issue for their clinical application as they may lead to spurious risk estimates in some individuals.

$$MISSING OR BAD TABLE SPECIFICATION {DDDC9DC5-2D36-492C-A3C6-1552022646C9}$$

Pasifika Heart Study cohort demographics	
N = 183	**N (%); median (IQR)**
Age (years)	40 (32–49)
Sex (male)	86 (47)
Samoan	82 (45)
Tongan	46 (25)
Fijian	41 (22)
Other Pacific	14 (8)
BMI (kg/m^2)	35 (30–40)
Current smoker	28 (15)
Type 2 diabetes	27 (15)
CVD at baseline	0 (0)
Fatal or non-fatal CV event	22 (12)
Follow up time (years)	8.3 (8.0–8.9)

Results of independent Cox models adjusted for baseline CVD risk			
	Hazard ratio (adjusted)	**95% CI**	**P-value**
Horvath (age-adjusted)	1.09	[0.97, 1.22]	0.14
Hannum (age-adjusted)	1.16	[1.02, 1.34]	0.039
PhenoAge (age-adjusted)	1.12	[1.02,1.23]	0.016
PACE (z-score)	1.53	[1.01, 2.35]	0.046
PoAm (z-score)	0.91	[0.60,1.38]	0.66

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P19.080.A Pleiotropic effects of genetics variants on Substance Use Disorder and Sleep Patterns

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Background: The neurobiology of substance use disorder (SUD) and sleep disorders are connected; however; little is known about the genetic mechanisms underlying their relationship.

Material and Methods: We have conducted a phenome-wide association study (PheWAS) in the São Paulo Epidemiologic Sleep Study (EPISONO, N = 1,042). The single nucleotide variants (SNV) rs4238213 and rs4727799 were selected from previous SUD genome-wide association studies. We tested the association between SNV genotypes and 460 EPISONO phenotypes, using age, squared-age, sex, a socioeconomical index and principal components (PCs 1–10) as covariates. Non-parametric tests were applied to validate nominally significant associations. To correct for multiple testing, we applied Bonferroni correction, treating each trait as a test, independently for each SNV ($p = 0.05/460$). OpenGWAS R package was applied to search for the potential associations of SNVs with traits available in other cohorts.

Results: We found SNVs' nominally significant associations for 36 phenotypes, including traits related to substance use and sleep patterns. SNV rs4238213 was nominally associated with lower odds of tobacco use (OR = 0.78) and increased REM sleep latency ($\beta = 0.10$). rs4727799 associations included higher odds of tobacco use (OR = 1.61) and decreased chance of drowsiness while reading (OR = 1.37). Traits related to sleep-disordered breathing were implicated with both SNVs. In the OpenGWAS database search, rs4727799 showed potential associations with sleep duration and chronotype in UK Biobank.

Conclusion: Our results suggest a pleiotropic effect of both SNVs on SUD and sleep-related traits, highlighting the possibility of an overlap between the genetic factors involved in the risk for SUD and sleep phenotypes.

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P19.081.B Secondary Findings in 81 ACMG Recommended Genes: Insights and Healthcare Implications in 1,000 Greek Exomes

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Background: Exome/Genome Sequencing (ES/GS) often uncovers additional clinically actionable genetic variations, termed Secondary Findings (SF). The American College of Medical Genetics and Genomics (ACMG) recommends analyzing 81 (v3.2) specific genes linked to conditions where early intervention significantly improves outcomes, including hereditary cancers, cardiomyopathies, and metabolic disorders. In this Greek cohort of 1000 exomes, secondary findings in the recommended genes are addressed in the context of both frequency of pathogenic or likely pathogenic variants and healthcare management.

Material and Methods: Pathogenic Single Nucleotide (SNVs) and Copy Number Variants (CNVs) were assessed using the 5% minor allele frequency threshold, ClinVar submissions, and ACMG classification criteria. Variants contributing to phenotype-causing

genotypes, in corresponding patients of the cohort, were not included.

Results: A total of 86 variants in 29/81 genes were detected in 118 samples (12%). Of these findings, 61% were associated with autosomal dominant phenotypes and 39% with autosomal recessivephenotypes. Variants in cardiovascular phenotypes were 37%, following from variants related with hereditary cancers in 23% of which 40% were associated with breast and ovarian cancer. In addition, 25 samples were carriers for the Wilson syndrome. Genes with the highest variant rates include *BTD, ATP7B, TTN, MUTYH* and *RYR1*.

Conclusion: Our results highlight the value of ES/GS not only in the diagnosis of rare diseases, but also in identifying clinically actionable SF. Providing insights into the burden of actionable genetic variants in the Greek population, can help informed clinical practices and guide the implementation of precision medicine initiatives.

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P19.082.C Geographic and Molecular Characterization of Duchenne Muscular Dystrophy in Ecuador: Insights from the National Registry

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Background: Duchenne Muscular Dystrophy (DMD) is a progressive, degenerative, and disabling genetic disorder affecting primarily pediatric populations. In Ecuador, the National DMD Registry offers valuable data for analyzing the geographic distribution, genetic, and clinical characteristics of patients, allowing for meaningful comparisons with international populations. The registry also assists in identifying inequalities in access to diagnostic and treatment services.

Material and Methods: A secondary data analysis was performed using the Ecuadorian Registry of Muscular Dystrophy, focusing on 90 confirmed DMD cases. The analysis identified the most frequent pathogenic polymorphisms and assessed geographic clusters using Geographic Information Systems (GIS) tools. The molecular findings were compared with global studies on the prevalence of deletions, duplications, and point mutations.

Results: Point mutations were identified in 49.35% of cases, with c.9091G>T, c.6982A>T, c.9100C>T, and c.9802C>T being the most frequent, each with a 2.63% relative frequency. Deletions accounted for 42.86%, primarily in exons 44 and 45, consistent with global patterns. Duplications were found in 7.79% of cases. Furthermore, 22% of patients were receiving combined treatments, including corticosteroids and ataluren. However, access to specialized care was limited to certain regions, indicating significant geographic disparities in healthcare availability.

Conclusion: This study highlights recurrent molecular patterns in Ecuadorian DMD patients, with a notably higher prevalence of point mutations compared to international data. The geographic disparities in diagnostic and treatment access underscore the urgent need to strengthen genetic health services, ensuring equitable and effective care for DMD patients in Ecuador.

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P19.084.A Epigenetic profiling of severe asthma patients treated with biological therapies

Giada Moresco[1], *Laura Fontana*[1,2], *Elena Maria Parazzini*[3], *Stefano Quaranta*[1], *Ornella Rondinone*[1], *Marzia Gianò*[1], *Enrico Zoroddu*[4], *Beatrice Re*[3], *Cristina Albrici*[3], *Cecilia Rosso*[5], *Francesco Ferella*[5], *Matteo Floris*[4], *Carlotta Pipolo*[5], *Giovanni Felisati*[5], *Michele Mondoni*[3], *Stefano Centanni*[3], *Monica R. Miozzo*[1,2]

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Background: About 30 million people worldwide are affected by severe asthma, a chronic respiratory disease with persistent symptoms despite glucocorticoid therapy. Approved biological treatments, including Mepolizumab, Benralizumab, and Dupilumab, inhibit type-2 cytokines (IL-5, IL-4, IL-13) binding to respective receptors and are highly effective in reducing symptoms. However, their precise mechanism of action is not yet well known, and there are no molecular biomarkers for predicting/monitoring treatment efficacy to assist clinicians in choosing the best therapy. We focused on methylation signatures related to asthma pathogenesis and biological treatments, to identify molecular biomarkers for personalized therapy.

Material and Methods: We analyzed the genome-wide methylation profiles of the nasal epithelium collected from 4 controls and 11 severe asthma patients, before therapy initiation (T0) and after 6 (T1) and 12 months (T2) of therapy. Libraries were prepared using the Diagenode Premium RRBS V2 kit and sequenced on the Illumina Nextseq2000 instrument. Data analyses were performed using homemade pipelines to identify DMRs and enriched pathways.

Results: Significant hypermethylation was evident in the nasal epithelium of severe asthma patients, and therapy-induced methylation changes correlated with improved clinical outcomes (mainly with Dupilumab at T1). Genes belonging to immune (*GATA3*, *ALOX5*), neuronal (*DLGAP1*, *CNTNAP2*), and chromatin remodeling (*WDR5*, *ARID3A*) pathways emerged as differentially methylated, suggesting their relevance in asthma pathogenesis and treatment response. Further sequencing and expression analyses of candidate genes are ongoing.

Conclusion: Methylation signatures could represent novel biomarkers for patient stratification, personalized therapy, and to monitor treatment efficacy.

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P19.085.B Clinical exome sequencing in 25 Moroccan pediatric cases: Impact, challenges, and lessons from a low- and middle-income country

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Background: Clinical Exome Sequencing (CES) has emerged as a groundbreaking tool for investigating rare genetic disorders and alleviating the associated "diagnostic odyssey" that has historically burdened patients and healthcare systems. However, the global adoption of CES remains uneven, particularly in Low- and Middle-Income Countries (LMICs) where genetic testing still faces numerous challenges.

Material and Methods: In order to assess the impact of CES, as well as the challenges and unique aspects of its application in a resource-limited environment, We studied a cohort of 25 Moroccan children suspected of having genetic disorders who underwent exome sequencing.

Results: CES yielded a diagnosis in 68% (n = 16) of the cases, significantly higher than the 30–50% diagnostic yield typically reported in the literature. The identified variants covered a broad spectrum of genetic conditions. Key challenges included a lack of awareness and understanding of CES among both patients and their primary healthcare providers, which was observed in 100% of the cases. Additionally, 40% (n = 10) of the cases presented incomplete or difficult-to-trace medical histories and prior diagnostic investigations, while 20% (n = 5) faced challenges in follow-up care.

Conclusion: Despite limited resources and infrastructure in the Moroccan healthcare system, CES provided critical diagnostic insights that were previously unattainable, demonstrating its capacity to overcome barriers and emphasizing the importance of efforts to democratize genetic testing technologies globally.

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P19.086.C Preliminary results from The PoliGeNReg Project: Inventory of registered ADPKD cases in a bicentric cross-border study

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a genetic disorder and the 4th leading cause of renal replacement therapy. Its prevalence is estimated at 1 per 1,000 in population-based studies and 2–4 per 10,000 in large healthcare databases, indicating that its true burden remains uncertain. The PoliGeNReg project is a two-year cross-border study in Romania and the Republic of Moldova, aiming to establish an ADPKD patient registry that integrates epidemiological, clinical, and genetic data to improve disease outcomes.

Materials and Methods: The PoliGeNReg project is developed in collaboration with Renal Disease Expertise Centers in North-Eeastern Romania and the Republic of Moldova. As a preliminary phase, data from ADPKD patient databases in both regions were retrospectively collected and analyzed.

Results: A total of 522 patients with ADPKD accessed medical care in the past five years, with 42.13% from the Republic of Moldova and 57.87% from Romania. The preliminary epidemiological analysis includes parameters such as sex distribution, age range, number of medical services accessed annually, and the most common complications. Further data analysis, including chronic kidney disease (CKD) staging, extrarenal manifestations and genetic findings is currently ongoing.

Conclusions: The PoliGeNReg project provides a structured framework for the epidemiological assessment of ADPKD in a bicentric cross-border setting. Preliminary results highlight the clinical heterogeneity of the disease and the need for standardized monitoring of CKD progression, extrarenal manifestations, and genetic profiles. Future efforts will focus on genetic testing, refined CKD staging, and improved long-term follow-up, contributing to better risk stratification and personalized treatment.

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P19.087.D Frequency of polymorphic variants associated with impaired estrogen metabolism in the Bulgarian population

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Background: The metabolism of estrogens leads to formation of 3,4-quinones which are major carcinogenic metabolites of estrogens. These compounds react with DNA, generating mutations that can accumulate and contribute to the development of neoplasia. The aim of this study is to determine the frequency of polymorphic variants associated with impaired estrogen metabolism in Bulgarian population.

Methods: Eighty volunteers were tested for twelve common genetic variants: *CYP1A1* (Msp1 T>C), *CYP1A1* (Ile462Val), *CYP1B1* (Val432Leu), *CYP17A* (34T>C), *MnSOD* (Ala16Val), *GSTM1* (Ins/Del), *GSTT1* (Ins/Del), *COMT* (Val158Met), *MTHFR* (677C>T), *SULT1A1* (Arg213His), *NQ01* (609C>T), *FACTOR V* (G1691A). All participants completed a questionnaire to assess dietary, epidemiological, and occupational risk factors that interact with the genome to predispose to disease development and progression.

Results: Preliminarily results of the 31 patients demonstrate that:-16 % are carriers of *CYP17A*, *COMT* and *GSTT1* risk variants;-58 % have deletion of *GSTM1* gene and 10% are double carriers of GSTM1/GSTT1 deletion;-16 % are carriers of three out of twelve high-risk polymorphisms;-9,6 % carry three high-risk and four moderate-risk variants;-25,8 % carry five to seven intermediate-risk polymorphisms, cumulatively affecting estrogen detoxification.

Conclusion: Specific combinations of high- and intermediate-risk genetic variants are common in the studied population. Genetic screening that considers the cumulative effect of multiple polymorphisms may provide a more comprehensive assessment of an individual's risk for impaired estrogen detoxification and the development of estrogen-dependent disorders.

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P19.088.A Causality between longitudinal adiposity changes and lung dysfunction

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Background: Lung function (LF) is a critical indicator of respiratory health and overall well-being. Obesity-induced LF changes involve mechanical alterations and inflammation driven by adipokines, with timing of obesity onset being crucial. However, the causal impact of longitudinal changes in body mass index (BMI) remains unclear. Previous research has not fully explored whether changes in body size over time influence LF parameters, including forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and their ratio (FEV1/FVC). This study aims to investigate the potential causal relationship between longitudinal body-size changes and LF.

Material and Methods: We conducted a two-sample Mendelian randomization (MR) analysis to assess the causality between longitudinal body-size change (LBC) and LF. LBC, as exposure, was derived from UK Biobank-based genome-wide association study (GWAS, n = 400,429, European ancestry), with association analyses performed using BOLT-LMMv2.4. LF outcomes (FEV1, FVC, and FEV1/FVC) were obtained from SpiroMeta consortium (Shrine et al.,2019;PMID:30804560).

Results: The Weighted Median method indicated a modest negative relationship between LBC and the FEV1/FVC ratio (beta[SE] = −0.19[0.095], p = 0.04). Potential causal relationship was observed between LBC and FVC using Weighted median method (beta[SE] = 0.19 [0.095], p = 0.04), both were nonsignificant after Bonferroni correction. We detected no causal effect of LBC on FEV1, and there was no evidence of pleiotropy.

Conclusion: Our findings suggest that LBC may have a modest impact on LF, particularly affecting FEV1/FVC and FVC. The overall relationship remains complex, highlighting the need for further research on the long-term effects of adiposity changes on respiratory health.

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P19.090.C Differentially expressed genes of human lens epithelial cells in different stages of diabetic retinopathy

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Background: Diabetic retinopathy (DR) and cataract are leading causes of blindness. This study aims to identify differentially expressed genes (DEGs) in human lens epithelial cells across various stages of DR to elucidate disease mechanisms, identify biomarkers, and discover potential drug targets.

Material and Methods: A cross-sectional comparative transcriptomics study was conducted with 250 participants recruited from a cataract waiting list at a public hospital in Malta. Participants underwent physical examinations, ophthalmic assessments, and blood tests (e.g., full blood count, renal/liver function, glucose levels). Lens capsule samples were collected during cataract surgery from non-diabetic controls (G1) and patients with no DR (G2), non-proliferative DR (G3), diabetic macular oedema (G4), and proliferative DR (G5). RNA sequencing of 50 samples (10 per group) was performed using the DNBSEQ platform, and GO and KEGG pathway analyses were conducted using the Dr. Tom bioinformatics system.

Results: A total of 173 DEGs were identified. Significant DEGs were determined using a log2 fold change threshold of ≥1.5 for upregulation and ≤−1.5 for downregulation, with a Q-value <0.05. Compared with controls, one downregulated DEG was identified between G1 and G2, 68 DEGs (32 upregulated, 36 downregulated) between G1 and G3, 43 DEGs (29 upregulated, 14 downregulated) between G1 and G4, and 61 DEGs (35 upregulated, 26 downregulated) between G1 and G5. Bioinformatics analyses revealed key DEGs and their associated pathways.

Conclusion: This study provides insights into molecular mechanisms underlying DR, age-related cataract and diabetic cataract, identifying potential biomarkers and therapeutic targets.

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P19.091.D Prevalence and Natural History of Allan-Herndon-Dudley Syndrome: Insights from a French Rare Disease Cohort

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Background: Allan-Herndon-Dudley Syndrome (AHDS) is a rare X-linked syndrome caused by pathogenic variants in the *SLC16A2* gene. The syndrome disrupts the transport of thyroid hormones into the brain, resulting in severe intellectual and motor disabilities in affected males. While over 300 affected males from 100 families have been reported, the exact prevalence remains unknown. Triiodothyroacetic acid (tiratricol) is currently under clinical trials. Limited knowledge of the natural history, the potential benefits of early intervention, and the absence of long-term follow-up data represent significant barriers to comprehensive care.

Material and Methods: This study leverages data from the French National Rare Disease Database to enhance epidemiological understanding of AHDS. Key parameters include patient prevalence, age distribution, follow-up status, and mortality rates. A dedicated electronic case report form (eCRF) was developed collaboratively with physicians and reference laboratories to capture longitudinal clinical and biological data, differentiating outcomes between treated and untreated patients.

Results: 56 individuals (52M/3F) with pathogenic *SLC16A2* variants have been identified with an estimated prevalence of <1 per million in France. The median age is 13 years (IQR 9–20). Ten patients have died, with a median age at death of 13 years. 13 individuals currently receive compassionate tiratricol therapy. The eCRF is scheduled for launch in 2025.

Conclusion: This study, grounded in a national epidemiological effort, addresses critical gaps in AHDS knowledge by collecting and comparing real-world data from treated and untreated patients. It aims to inform long-term follow-up, optimize patient care, and guide future therapeutic strategies for this disorder.

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P19.092.A Advancing genomic analysis to resolve the diagnostic odyssey of rare diseases in a developing country

. ***Gunadi***[1], *Kristy Iskandar*[2], *Dewiyani Widasari*[1], *Wulan Anggrahini*[1], *Syallom Gita Maharani*[1], *Pramana Adhityo*[1], *Nabila Ardia Pramono*[1], *Ali Zainal Abidin*[1], *Auliya Suluk Brilliant Sumpono*[1], *Yunika Puspa Dewi*[3], *Tyas Ikhsan Hikmawan*[4]

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Background: The underrepresentation of Asian genomes hinders the progress of genomic medicine, particularly in developing countries. Indonesia, with its diverse population and approximately 13 million individuals affected by rare diseases, faces significant challenges in diagnosing these conditions. Many patients experience lengthy diagnostic journeys, which prolong treatment. The Biomedical & Genome Science Initiative (BGSi), launched by Indonesia's Ministry of Health, seeks to tackle these challenges by establishing a genomic research infrastructure that includes Dr. Sardjito Hospital as the genomic hub for rare diseases. The initiative follows a comprehensive genomic analysis framework encompassing biobanks, patient registries, genomic analysis, bioinformatics, molecular diagnostics, and personalized therapy development.

Material and Methods: Patients with rare diseases were enrolled through outpatient clinics and hospital wards. After obtaining informed consent, patient data were registered, and blood samples were collected, stored in our institution's and national biobank, and processed for DNA extraction. Exome sequencing was performed using the NextSeq 550 platform, while adaptive sampling was utilized for 200 targeted panel genes using PromethION technology.

Results: In 2024, 642 patients with a rare disease were recruited. We analyzed the exome sequencing of the DNA from 9 patients using the NextSeq 550 and conducted adaptive sampling of the DNA from 6 patients using the Promethion. After receiving the results, some diagnoses were changed, and the treatments were updated accordingly.

Conclusion: By employing a genomic approach, we revolutionize the diagnosis of rare diseases, establishing a foundation for precision medicine through improved genomic research infrastructure and national collaboration.

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P19.093.B Ethnic differences in the frequency of *CFTR* gene variants in the Russian Federation

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Background: Russia is a home to more than 190 ethnic groups, including small indigenous and autochthonous nations of the country. Cystic fibrosis (CF) is a genetically inherited disorder characterized by a wide range of clinical manifestations and genetic variations.

Material and Methods: Genetic testing of healthy population and people with CF (pwCF) of different parts of Russia.

Results: The most common *CFTR* variants among pwCF in Russia: F508del (51.44%), CFTRdele2,3 (6.10%), E92K (3.71%), 1677delTA (2.51%), 3849 + 10 kbC-T (2.24%), 2143delT (1.995), 2184insA (1.93%0, W1282X (1.73%), L138ins (1.71), N1303K (1.62%), G542X (1.47%). High population frequency of **E92K** was found in the Turkic-speaking groups of the Volga region (VR) and North Caucasus (NC): Chuvash (53.12%), Chechens (12.5%), Udmurts (6.8%), Tatars (2.4%); **W1282X** - in Turkic-speaking groups: Karachais (90%), Nogais (100%), Abazins (50%) and Ossetians (37.5%); **1677delTA** - in autochthonous peoples as the Abkhaz-Adygean (Abazins and Circassians over 30%) and Nakh groups: Chechens (81.3%) and Ingush (100%). It has also been detected in other ethnic groups from the NC region, such as Armenians (25%) and Georgians (25%). Since 2007 the National Neonatal Screening programme (NSP) for CF has been introduced. Interestingly, even considering the NSP, no pwCF have been identified so far in the Yakut and Kalmyk populations.

Conclusion: Interethnic differences in the spectra of *CFTR* gene variants were shown both in pwCF and in healthy population of different parts of Russia. The most significant variation was identified in variants E92K, 1677delTA, and W1282X. Further studies are necessary in different populations.

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P19.094.C No evidence for a causal effect of thyroid function and disease on risk for attention- deficit/hyperactivity disorder: A two-sample Mendelian randomization study

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Background: Attention-deficit/hyperactivity disorder (ADHD) is the most prevalent neurodevelopmental disorder among children, characterized by symptoms of inattention, hyperactivity, and impulsivity. These symptoms can overlap with those observed in

individuals with thyroid disorders, especially hyperthyroidism. Previous epidemiological and clinical studies have indicated an association between thyroid function and dysfunction and ADHD. However, these studies may be subjected to different sources of confounding.

Material and Methods: Mendelian randomization (MR) was employed to overcome the limitations of previous association studies, particularly reverse causation and residual confounding. The instrumental variables for thyroid stimulating hormone (TSH), circulating free T3 (FT3), and circulating free T4 (FT4) were derived from a genome-wide association study (GWAS) of 271,040 individuals of European ancestry. For hypo- and hyperthyroidism, Hashimoto's thyroiditis, and Graves' disease, a cross-population GWAS was used (1.216 to 15.434 cases, up to 630.000 controls). For ADHD, we used the most recent GWAS with 38,691 cases and 186,843 controls. To account for possible violations of MR assumptions, we performed two-sample MR using eight different methods.

Results: We found no causal effect of any thyroid function phenotype on the risk of ADHD.

Conclusion: Although ADHD and thyroid dysfunction have overlapping symptoms, there was no evidence of a causal relationship. However, the power to draw definitive conclusions regarding hyperthyroidism and Grave's disease was limited. Nevertheless, these data do not encourage routine testing of thyroid function in cases with ADHD in the absence of additional symptoms suggestive of thyroid dysfunction.

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P19.095.D Identifying Potential Drug Targets for Brain Tumors through Mendelian Randomization Analysis

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Background: Brain tumors are a major health threat with poor prognosis, and identifying effective drug targets for treatment is crucial. Mendelian randomization (MR) analysis has emerged as a powerful tool for discovering causal relationships between genetic variations and diseases. This study aimed to use MR methods to identify potential drug targets for brain tumors.

Material and Methods: We performed summary data-based Mendelian randomization (SMR) analysis, colocalization analysis, and two-sample Mendelian randomization analysis using data from genome-wide association studies (GWAS) and cis-expressed quantitative trait loci (cis-eQTL) databases. Sensitivity analyses were carried out to ensure the reliability of the findings.

Results: The analysis identified four potential drug targets for brain tumors: *FAM160B1*, *GMEB2*, *RP11-617D20.1*, and *TBC1D7*. *FAM160B1* was found to be a risk factor for brain tumors (odds ratio [OR] > 1), while *GMEB2*, *RP11-617D20.1*, and *TBC1D7* were identified as protective factors (OR < 1). These genes may serve as novel targets for therapeutic intervention.

Conclusion: This study identified four potential drug targets for brain tumors through Mendelian randomization analysis. These findings provide valuable insights for drug development and treatment strategies, with potential clinical applications for brain tumor therapy.

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P19.096.A Plasma proteome-based prediction for COVID-19 vaccination response

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The COVID-19 pandemic has posed a significant global health crisis, and although vaccines have been developed, the variability in individual immune responses remains poorly understood. Predicting vaccination outcomes before administration could help to optimize vaccine strategies for individuals. This study involved follow-up blood sample collection from 208 Chinese participants who received three doses of the inactivated COVID-19 vaccine. Using association studies and Mendelian randomisation (MR) analysis of proteins with changes in COVID-19 antibody levels, we identified 14 biomarkers significantly associated with the responses post-vaccination. Before vaccination, a protein-level predictive model could forecast vaccine effectiveness for the primary series ($r^2 = 0.174$), booster shot ($r^2 = 0.131$), and complete vaccination series ($r^2 = 0.114$). Notably, polygenic scores (PRS) derived from these proteins and COVID-19 infection traits also provided predictive ability. In particular, COVID-19 infection traits outperformed protein scores in predicting responses after the primary series and booster shot, while protein scores were more effective in predicting antibody changes following the complete vaccination series. This study highlights the potential of plasma proteome-based models and polygenic scores to predict COVID-19 vaccination outcomes, offering insights into the mechanisms of immune response variability and aiding in the development of personalized vaccination strategies.

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P19.097.B Evolution of situations of diagnostic deadlock and impasse considering the integration of new technologies in French healthcare from 2012 to 2022: A study of the AnDDI-Rares network within the Diagnosis Observatory

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Background: The 3rd French National Plan for Rare Diseases focuses on reducing diagnostic. The AnDDI-Rares network implemented a project to assess diagnostic evolution in France over a decade (2012–2022) with the integration of high-throughput sequencing technologies.

Material and Methods: This study analyzed diagnostic rates in French centers for "Developmental Anomalies and Malformative Syndromes," comparing randomly selected weeks in 2012 and 2022. Secondary outcomes included diagnostic delays, patient expectations, technologies used and genome sequencing efficiency.

Results: The number of patients included was 303 in 2012 and 350 in 2022, representing an 15.5% increase in clinical genetic activity in France over ten years. The diagnostic rate for patients coming for a first advice increased from 18.6% to 23.9%. Overall genome diagnostic yield was 28.8%. Among 57 undiagnosed patients from 2012 who expressed a desire to continue investigations, genome sequencing yielded an additional 33.3% diagnostic rate. The average waiting time for positive results decreased from a median of 3 years in 2012 to 1.0 years in 2022. In total, 164 parents completed questionnaires on diagnostic expectations, revealing evolving patient needs and attitudes over time. Significant heterogeneity in diagnostic strategies was observed across centers, highlighting the impact of genome sequencing.

Conclusion: These results highlight advancements in diagnostic practices and the pivotal role of high-throughput sequencing, supported by the France Genomic Medicine 2025 Plan. The challenges of recontacting patients after 10 years are evident. Persistent diagnostic requests and insights from long-term follow-up underscore the need for continuous patient engagement and tailored support strategies.

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P19.098.C Mendelian Randomization approach to unraveling the genetic link between eating behavior and metabolic traits

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Background: Disordered eating contributes to weight gain, obesity, and type 2 diabetes (T2D), yet the mechanisms linking eating behaviors to specific metabolic phenotypes remain unclear.

This study aims to identify genetic variants associated with eating behaviors and examine their causal relationships with metabolic traits using Mendelian randomization (MR).

Material and Methods: We investigated the association between genetic variants in 30 genes and eating patterns in 200 individuals with T2D f and 397 healthy controls from the Volga-Ural region. Eating behaviors were assessed using the Dutch Eating Behaviour Questionnaire (DEBQ), categorizing eating patterns into emotional, external, and restrained eating. Two-sample MR analyses were performed to assess causality between eating behaviors and metabolic traits, utilizing summary statistics from large international consortia, including the UK Biobank, MAGIC, and the Global Lipids Genetics Consortium.

Results: We identified significant genetic associations between specific polymorphisms and eating behaviors. Notably, *HTR2A* rs6313 was associated with emotional eating, and *HTR1D* rs623988 and *CDKAL1* rs9295474 with external eating. MR analysis showed that external eating was inversely associated with glycated hemoglobin (HbA1c) in people with metabolic syndrome (MetS) and was linked to various anthropometric traits in the GWAS cohorts, such as BMI, waist circumference, and hip circumference.

Conclusion: Our findings suggest a genetic basis for different eating behaviors and their influence on metabolic traits. External eating, in particular, was found to have a causal relationship with glycemic and anthropometric traits. These results highlight the importance of genetic factors in shaping eating behaviors and their potential impact on metabolic health.

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P20 Pharmacogenomics and Drug Repurposing

P20.002.A A Transcriptome-Wide Association Study to uncover the genetic factors driving toxic responses after chemotherapy with fluoropyrimidines in colorectal cancer

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Background: Colorectal cancer (CRC) is a deadly malignancy often requiring surgery and chemotherapy. Standard treatments, including fluoropyrimidines (5-fluorouracil or capecitabine) with oxaliplatin or irinotecan, improve survival but often cause adverse drug reactions (ADRs), affecting outcomes. Pharmacogenomics optimizes therapy by identifying genetic variations influencing drug efficacy and toxicity. While genome-wide association studies (GWAS) have had limited success, transcriptome-wide association studies (TWAS) integrate transcriptomic data with GWAS to discover new risk loci.

Material and Methods: Using summary statistics from the QUASAR2 pharmacogenomics GWAS (one of the largest toxicity GWAS reported), we conducted TWAS with PrediXcan to identify genomic loci associated with chemotherapy-induced toxicity across 50 tissue types. We performed colocalization with fastENLOC and fine-mapping through FOCUS to explore gene causality. Overlapping genes from TWAS and fine-mapping were prioritized for functional analysis. Additionally, we developed a Polygenic Transcriptome Risk Score (PTRS) to categorize patients based on their individual toxicity risk.

Results: We discovered 157 genomic loci significantly associated with toxicity risk through regulation of the expression of 187 candidate genes. Our fine-mapping analyses identified 11 of these genes as causal to ADR development. The derived PTRS weights demonstrated potential for stratifying populations based on their genetic predisposition to chemotherapy toxicity.

Conclusion: Integrating transcriptomic data into pharmacogenomic has allowed us to significantly enhance the identification of genetic risk loci associated with chemotherapy-induced toxicity in CRC. Moreover, our transcriptome-based analyses can contribute to gaining a deeper understanding of the molecular mechanisms underlying ADRs and support the development of personalized therapeutic strategies for CRC patients.

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P20.003.B Pharmacogenetic analysis from exome sequencing data, combined with pharmaco-clinico-biological co-interpretation facilitates personalized management in nephrology and kidney transplantation

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Background: Preemptive pharmacogenetic panel testing has been shown to reduce the incidence of clinically detectable side effects by 30%. At Sorbonne University, exome sequencing is routinely performed for adults with genetically-suspected Chronic Kidney Disease (CKD), offering not only molecular diagnosis but also underutilized pharmacogenetic insights. CKD patients, who are frequently polymedicated, could greatly benefit from pharmacogenetic data as treatment efficacy and safety are influenced by genetic factors.

Material and Methods: We analyzed pharmacogenetic profiles for 456 adult patients who underwent exome sequencing (Twist capture, Illumina NovaSeq6000, 2×150 bp) for unknown nephropathy. The analysis targeted 217 variants in 23 pharmacogenes following CPIC and DPWG guidelines. HLA-A and HLA-B loci were characterized using a dedicated pipeline. Star alleles translation and dosing guidelines were conducted using SONOGEN-XP (INTLAB AG). 68 patients received medication reconciliation and counseling to assess potential dosage adjustments or treatment modifications. 19 cases were reviewed in multidisciplinary consultation meetings (MCM).

Results: In-house pharmacogenetic analysis demonstrated complete concordance with reference samples from Get-RM and orthogonal methods (PCR amplification and LC-MS detection, Immucor for HLA profile study). 100% of patients had at least one actionable pharmacogene variant. Following MCM, all 19 patients received recommendations regarding drug dosing or contra-indications, totaling 60 individualized recommendations. Relevant drugs impacted by altered metabolism included tacrolimus (9/19), azathioprine (1/19), tramadol (6/19), clopidogrel (7/19), allopurinol (3/19), atorvastatin (5/19).

Conclusion: Pharmacogenetic analysis based on exome is a valuable tool in CKD management, helping prevent adverse drug effects. It is particularly beneficial for patients with forthcoming immunosuppressive therapy for kidney transplantation.

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P20.004.C Polygenicity of the corticosteroid response in patients with severe infections

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Background: Corticosteroids (CS) improve survival in severe infections, but variable responses have been reported. We aimed to identify genetic variants that could be associated with the differential CS response.

Material and Methods: We performed a genome-wide association study (GWAS) of 90-day mortality in a cohort of hospitalized COVID-19 patients treated with CS from the SCOURGE consortium (Ncases = 375, Ncontrols = 1,850). Logistic regression models were conducted in ~9.5M TOPMed-imputed variants (significance set at $p = 5 \times 10^{-8}$). Then, we tested the polygenic overlap of the CS response between COVID-19 and all-cause sepsis using PRSice-2 to perform polygenic risk scores (PRS). The PRS were tested on the independent CS-treated all-cause sepsis patients (Ncases = 21, Ncontrols = 69) from the GEN-SEP cohort. The best PRS model was subject to pathway-specific PRS studies using PRSet to evaluate which pathways were related to the differential CS response.

Results: Three *PLCG2* variants, two intronic and one in the 3'-UTR region, were associated with CS response in COVID-19 ($p_{lowest} = 2.79 \times 10^{-8}$). A PRS model including 31,374 variants reaching a $p_{threshold} = 0.0298$ in the GWAS was nominally associated with CS response among all-cause sepsis patients ($p = 0.044$). Pathway-specific PRS analyses revealed the regulation of the cholesterol biosynthesis pathway as the most significantly associated with CS response in all-cause sepsis ($p = 0.001$).

Conclusion: Overlapping polygenic effects of the CS response between COVID-19 and all-cause sepsis were observed. Genetic variants affecting the regulation of cholesterol biosynthesis pathway could underlie the CS response differences during critical illness.

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P20.006.A OncoAID: an open access targeted anti-cancer database

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Background: The rapid expansion of targeted therapies, from the first FDA-approved agent to over 150 by 2024, underscores the growing need for comprehensive and accessible oncology resources. Existing databases often lack global regulatory perspectives and integrated clinical data, limiting the scope of precision oncology. To this end, we developed OncoAID, an open-access database of FDA-approved anti-cancer therapies, enriched with external datasets, global approvals and AI-driven summaries.

Material and Methods: OncoAID was curated by manually compiling a table of FDA-approved targeted therapies (single agents and combinations) from NIH and DRUGS.com with a few of their properties. Additional references to external databases (PHARMGKB, DrugBank, and TTD) were added to provide supplementary data. Global regulatory statuses (EMA, PMDA, NMPA, WHO) were verified through documented approvals and AI-assisted translations. Progression-free survival (PFS) and overall survival (OS) were extracted from the FDA and DRUGS.com, while cell line IC50 values were sourced from GDSC. Concise AI-generated (Chat GPT and Perplexity) summaries were also added.

Results: As of November 2024, OncoAID includes 576 regimens (303 unique treatments across 345 indications). Among 407 entries with PFS data, combination therapies significantly prolonged median PFS versus single agents (p = 0.0059). Approvals increased markedly, especially for combinations, and a Venn analysis revealed regulatory discrepancies among agencies.

Conclusion: By integrating global approvals, clinical outcomes, and experimental efficacy data, OncoAID offers a robust platform for precision oncology. Its open-access interface and AI-generated summaries support data-driven decisions. Ongoing expansions aim to refine AI validation, broaden coverage, and maintain continuous updates. Access at https://fohs.bgu.ac.il/rubinlab/oncoaid/.

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P20.007.B Defining the genetic determinants of CD8+ T cell receptor repertoire in immune checkpoint blockade

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Background: CD8+ T cells are central to cancer response during immune checkpoint blockade (ICB) therapy, with their activity driven by antigen recognition via the T cell receptor (TCR) repertoire. However, the role of genetic variation in shaping this repertoire and influencing treatment outcomes remains underexplored.

Material and Methods: Here, we genotyped 250 cancer patients and profiled their CD8+ T cell TCR repertoire before and after ICB treatment to explore the dynamic relationship between genetic variants and TCR repertoire. We conducted a genome-wide and human leukocyte antigen (HLA)-focused analysis of CD8+ T cell TCR repertoire using a linear regression model correcting for genetic principal components (PCs), TCR PCs, age, gender and cancer type.

Results: In total, we identified 11 genome-wide significant cis associations and 10 trans associations, primarily to the HLA, with V-gene usage meeting permuted P-value thresholds. Notably, TCR clones containing V-genes associated with HLA were less likely to be persistent across treatment. In a single-cell experiment, we find cells with HLA-matched TCR clones have increased tumor reactivity expression profiles and patients with HLA-matched TCR clones have improved overall survival.

Conclusion: Our work indicates a complex interplay between genotype and TCR repertoire in the context of ICB treatment, which has novel implications for understanding determinants of treatment response and patient outcomes.

C.O.I. Disclosure:

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P20.008.C From biobanks to bedside: Utilizing genetic data of population biobanks to prevent life-threatening adverse drug reactions

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Background: Adverse drug reactions (ADRs) constitute a major issue in health care settings, as exemplified by life-threatening reactions such as malignant hyperthermia (MH) in response to anesthetic drugs. While improvements in pharmacogenetics (PGx) research have revealed genetic risk factors for ADRs, the application of preventative PGx testing is currently limited. For this project, we aimed to use existing resources of population biobanks to identify individuals at risk of severe ADRs.

Material and Methods: We linked genotypes from individuals in the Danish Blood Donor Study (DBDS) (n = ~130.000) and Copenhagen Hospital Biobank (CHB) chronic inflammatory disease cohort (n = ~300.000) with Danish health care registries to integrate genotypes with diagnoses, hospitalizations and prescriptions.

Results: We confirmed a previously known association between rare variants in the RYR1 gene and the occurrence of MH (p = 6e-06). Importantly, we identified 23 individuals with RYR1 variants who did not have an MH diagnosis, potentially because DBDS participants, as healthy blood donors, have limited exposure to drugs triggering MH. Uniquely for DBDS participants, we have permission and a plan to contact individuals that we expect are unaware of their high genetic risk of MH.

Conclusion: This study proves that biobank-driven PGx testing could be an important tool for informing individuals about their genetic risk for ADRs. However, widespread application of PGx testing in biobanks is currently limited by legislation and ethical concerns. Legislators and researchers alike should move forward with discussions on whether using existing biobank data for PGx testing is not only a possibility, but also our ethical responsibility.

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P20.009.D miRNAs target of MEG3 lncRNA could modulate the response to biological treatments in Psoriatic Arthritis patients

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Background: Psoriatic arthritis (PsA) is a chronic inflammatory rheumatic disease. Although the identification of the correct therapy remains a critical issue, the introduction of biological drugs seems promising. Treatment response could also be affected by epigenetic factors, including long non-coding RNAs (lncRNAs). In fact, our recent paper on PsA patients suggested a possible involvement of the lncRNA MEG3 in the treatment response to two biological drugs, TNF- and IL17-inhibitors.

Material and Methods: To further investigate the possible role of MEG3 in treated PsA patients, we performed a bioinformatic analysis using different web-based miRNAs target prediction tools. Then, we analysed the expression levels of the identified miRNAs in a cohort of 54 PsA patients at the beginning of therapy (T0) and after 12 months (T12), in patients who responded to treatment. We also evaluated a possible association between *MEG3* genetic variants and the levels of its predicted miRNAs target.

Results: The prediction analysis identified four miRNA targets: miR-21-5p, miR-19b-3p, miR-19a-3p and miR-17-5p. Preliminary results showed that miRNA target expression levels do not differ between Responder and non-Responder patients at T0. However, in the group of Responder patients, we observed a significant decrease of miR-17 (P = 0.03) and miR-19b (P = 0.04) levels at T12 follow-up compared to T0. Moreover, we observed that rs941576 variant in *MEG3* gene seems to influence the expression levels of miR-19a (P = 0.04) and miR-19b (P = 0.005).

Conclusion: Our results suggest that MEG3 could modulate the efficacy of biological therapies in PsA patients acting on its miRNA targets.

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P20.010.A Investigating the genetic factors of medication dosing using biobank-linked drug purchase data

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Background: Despite significant pharmacogenomics (PGx) insights into key biological pathways influencing drug response, the polygenic contribution to dose variability and the potential of electronic health records (EHRs) for maintenance dose estimation remain largely unexplored. To address this gap, we leveraged longitudinal drug purchase data (median follow-up 7.3 years) from Estonian Biobank to derive medication dose metrics and investigate genome-wide associations.

Methods: We analyzed statins, warfarin, metoprolol, antidepressants, and antipsychotics, deriving daily doses per purchase, and median and maximum doses as consolidated metrics across purchases. Associations with polygenic scores (PGSs) for 16 traits were assessed using linear mixed models and multivariable regression with forward stepwise approach and Bonferroni correction. Genome-wide scans (GWAS) were followed by gene set enrichment analyses for PGx genes.

Results: Sample sizes ranged from 684 (antipsychotics) to 20,642 (statins), with median doses reflecting typical maintenance doses. PGS for indicator trait was significant for statins (coronary heart disease PGS; beta = 0.02, $P = 5.9 \times 10^{-10}$) and metoprolol (systolic blood pressure PGS, beta = 0.03, $P = 7.5 \times 10^{-13}$). Body mass index PGS was linked to daily doses of statins (beta = 0.02, $P = 6.4 \times 10^{-7}$), metoprolol (beta = 0.03, $P = 1.4 \times 10^{-14}$), and warfarin (beta = 0.03, P = 0.001), whereas educational attainment PGS showed opposing associations with statins (beta = −0.03, $P = 5.8 \times 10^{-4}$) and antidepressants (beta = 0.01, P = 0.002). Median and maximum doses showed similar but weaker associations. GWAS confirmed signals for metoprolol (*CYP2D6*, $P = 6.3 \times 10^{-21}$) and warfarin (*CYP2C9*, $P = 8.9 \times 10^{-60}$; *VKORC1*, $P = 4.2 \times 10^{-148}$), and gene enrichment revealed PGx relevance for statins (P = 0.006).

Conclusion: This study illustrates feasibility of deriving medication doses from EHRs and highlights independent contributions of polygenic liability and PGx factors to dose variability.

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P20.011.B *PCSK9* Variants and Their Impact on Glucose Metabolism and Type 2 Diabetes Susceptibility

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Background: Lipid-lowering medications, particularly statins (HMG-CoA reductase inhibitors), have significantly reduced cardiovascular events and mortality but are linked to an increased risk of type 2 diabetes (T2D). Similarly, inactivating mutations in *HMGCR* are associated with T2D. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, which effectively lower cholesterol, have yielded conflicting data on their role in glucose metabolism and T2D risk. Exploring genetic factors may provide valuable insights to inform clinical practice.

Material and Methods: This study examined the relationship between 226 *PCSK9* missense variants and metabolic traits, including LDL cholesterol, random glucose, and T2D, in 469,835 individuals from UK Biobank with whole-exome sequencing data. Statistical methods included single-variant analysis (PLINK), SKAT-O test, and unsupervised hierarchical clustering to identify potential variant groups of missense variant phenotypic effects.

Results: Single-variant analysis revealed significant associations between rs374014696 (Ala242Val, OR[95%CI] = 0.51[0.40–0.65], *p-value* $= 1.022 \times 10^{-7}$) and rs773660398 (Ala44Thr, OR: 1.50 [1.26–1.78], *p-value* $= 6.735 \times 10^{-6}$) with higher random glucose levels. Clustering analyses identified distinct variant groups. Cluster 1 variants were linked to lower LDL cholesterol and higher glucose. Cluster 2 variants were associated with lower T2D risk, while Cluster 3 showed a combination of lower LDL, higher glucose and T2D risk. Cluster 4 was associated with higher LDL cholesterol and likely elevated cardiovascular risk.

Conclusion: These findings suggest that certain *PCSK9* missense variants are associated with cholesterol reduction, higher glucose levels, and higher T2D risk. Genetic profiling may optimise lipid and glucose management strategies, emphasising the need for further studies in diverse populations.

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P20.012.C The genetics of treatment resistant depression in the Pakistani DIVERGE cohort

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Background: Treatment resistant depression (TRD) is responsible for half of the disease burden. Research on TRD from low-and-middle income countries is scarce. We take advantage of the deep phenotyping in DIVERGE, the first large genetic epidemiological study of depression in Pakistan (N = 13,000), to investigate risk factors for TRD.

Material and Methods: TRD was defined as minimal to no symptom relief after antidepressant treatment lasting at least 12 weeks and with evidence of good/moderate medication adherence. Illumina's Global Diversity Array was used for genotyping and the DRAGEN array caller to derive CYP2D6 and CYP2C19 metabolizer groups. Factors were tested for association with TRD using multilevel logistic regression.

Results: Using data from 3,677 eligible patients (34 % with TRD), we found that social support and parents being first cousin were associated with lower risk (OR = 0.56, p = 0.001, OR = 0.81, p = 0.016, respectively) whilst psychotic symptoms and suicidal behaviour were linked to higher risk of TRD (OR = 1.35, p = 0.04, OR = 1.02, p = 0.029). In 1,085 patients with CYP enzyme phenotype data, poor (OR = 1.7, p = 0.02) and ultra-rapid (OR = 3.27 p = 0.001) metabolizers for *CYP2C19* were associated with TRD. A pilot GWAS of TRD in 1,500 patients yielded three loci associations at suggestive threshold p < 5 × 10-5 including

rs2392034 with nearby ST6GALNAC3 relevant to neuronal biology and cell integrity.

Conclusion: Studying diverse populations is important to identify population specific risk factors for TRD. This study was successful in identifying preventable risk factors and supports the importance of pharmacogenetic biomarkers in predicting TRD.

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P20.013.D Exogenous hormones and cardiovascular disease risk: Decoding the molecular mechanisms of menopausal therapy

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Background: Menopausal hormone therapy (MHT) and its association with cardiovascular disease (CVD) is a concern for many women. Recently, we published the most comprehensive study on contemporary MHT use and CVD risk utilizing Swedish national registers. We identified that various MHT formulations, including combined estrogen-progestin therapies, estrogen-only, and tibolone and different routes of administration, such as oral and transdermal, exhibit variable effects on CVD risk[1]. However, the underlying molecular mechanisms remain unclear.

Methods: To identify molecular mediators responsible for the increased risk of stroke and myocardial infarction during MHT use, we conducted a two-stage study. First, we identified proteomic changes associated with MHT use among 16,000 post-menopausal women in the UK Biobank. Second, we established the causal link between proteins and disease risk using a two-sample Mendelian randomization approach.

Results: Out of 2,924 proteins measured, 821 were associated with at least one type of MHT, with the highest number for orally administered products containing estrogen. For stroke, we identified seven proteins (FDR $q < 0.05$) that mediate the effect of MHT, including F11, a key protein in the coagulation system. For myocardial infarction, we identified six proteins, including CD34 previously linked to atherosclerosis and myocardial infarction.

Conclusion: The strong association between orally administered MHT products and both protein expression and CVD risk[1] is likely mediated through first-pass metabolism of these products. Identifying the proteins that mediate the effect of MHT on CVD could reveal new potential targets for intervention to mitigate the CVD risk associated with MHT use.

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P20.014.A Regulatory polymorphisms of *MSH6*, *MSH2* and *PPP1R21* genes affect survival of immunotherapy-treated lung cancer patients

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Background: Immune checkpoint inhibitors (ICIs) have improved survival in non-small cell lung cancer (NSCLC) patients, however many patients do not respond to treatment. Identifying markers of ICI response remains an unmet clinical need. This study hypothesizes that host genetics influence the response to ICIs, contributing to variability in efficacy among individuals

Material and Methods: We conducted a genome-wide association study (GWAS) in patients with NSCLC receiving ICI monotherapy with nivolumab, pembrolizumab, or atezolizumab, to identify germline variants associated with objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) at 24 months after initiation of ICI therapy. Association analyses were performed for ORR (logistic regression using PLINK2 software) and survival (Cox proportional hazards model, using GenAbel package in R environment), with appropriate covariates

Results: We identified a locus on chromosome 2 (comprising more than 250 variants) associated with OS, with the top ten variants having a hazard ratio >1 and $P\text{-value} = 6.3 \times 10^{-8}$. The most significant polymorphisms were regulatory variants of genes at the locus, such as *MSH2*, *MSH6*, and *PPP1R21*, which play a role in the mismatch repair and endosomal trafficking, and may influence response to immunotherapy

Conclusion: This study identifies an association between a genomic locus on chromosome 2 and OS in NSCLC patients treated with ICI. Although these results require validation in larger cohorts and functional studies to elucidate the underlying mechanisms, they highlight the potential of germline variants as predictive biomarkers of response to ICI.

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P20.015.B 'Impact of Pharmacogenetics on the Personalization of Therapies for 100 Patients with Pulmonary Arterial Hypertension'

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Pulmonary arterial hypertension (PAH) is an infrequent and progressive disease characterized by sustained increased pressure in the pulmonary arteries, leading to high morbidity and mortality. Pharmacogenetics (PGx) enables personalized treatments through the analysis of genetic variants that affect the pharmacokinetics and pharmacodynamics of medications, thus optimizing the efficacy and safety of therapies.The aim of this study is to evaluate the impact of actionable genetic variants on the therapeutic response of PAH patients through a comprehensive pharmacogenetic analysis in a cohort of 100 patients from whole genome sequencing (WGS).Phased VCF files from PAH patients were analyzed using bioinformatics tools such as PharmCAT, StellarPGx, and xHLA. The analysis focused on key genes involved in the metabolism of level 1 medications, including warfarin and statins. Personalized PGx reports were generated, and the frequency of relevant variants in the cohort was evaluated.All patients presented between 7 and 60 PGx recommendations, with a mean of 28 recommendations per patient. For warfarin and statins, recommendations were obtained for 26.42% of the cohort. For statins, 31.74% of the recommendations were associated with ABCG2, CYP2C9, and SLCO1B1. For warfarin, 26.19% were linked to CYP2C9, CYP4F2, and VKORC1. The cis or trans configuration of haplotypes reinforced the accuracy of the recommendations.

This study demonstrates the high prevalence of actionable pharmacogenetic variants in patients with PAH, showing that integrating PGx into clinical practice can significantly improve therapeutic outcomes, consolidating its role in personalized medicine.

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P20.016.C Comparison of star-allele caller performances on genotyping and sequencing data at different geographic scales

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Pharmacogenetics is the study of genetic variants responsible for variable response to medication. These variants can explain alternate drug responses, and understanding their effects thus represents a key public health issue. To standardize pharmacogenetics studies, a specific nomenclature is used: the star-alleles, which associates haplotypes with the activity of a given pharmacogene. Such haplotype calling can be influenced by population origin, the complexity of the genomic region in question and choice of reference data; many star-allele callers have been developed with little insight on their performance in different scenarios.

The objective of this study is to determine the performances of the different tools available. To do this, we used sequencing data from the 1000 Genomes and FranceGenRef projects.

To this end, we have benchmarked different pipelines of star-allele annotation. We have observed some discrepancies between the output of star-allele annotation pipelines, coming from differences in the reference database used, the method employed and the range of each pipeline in terms of gene coverage and variant detection. We have also evaluated the impact of imputation on star-allele calling, examining its influence on calling accuracy and downstream phenotype prediction to assess the reliability of imputed data. We provide recommendations on which tools to use regarding the data available, the population observed and the gene studied.

This study provides insights into the strengths and limitations of different star-allele callers used for inferring unobserved data. These findings contribute to the assessment of the potential benefits of personalized care informed by pharmacogenetic variant information.

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P20.017.D Genetic Clustering of *GLP1R* Variants Reveals Diverse Impacts on Metabolism and Mental Health

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Background: Glucagon-like peptide-1 receptor (GLP-1R) plays crucial role in glucose metabolism and insulin secretion. GLP-1R agonists target GLP-1R while simultaneously bring changes to body weight and mental health, including depression. Missense variants in *GLP1R* gene change response to GLP-1R agonists by affecting the receptor structure and function, potentially leading to side effects. We investigated *GLP1R* gene variant's role in glucose metabolism, body mass index (BMI), and depression.

Methods: We analysed 72 coding missense variants in *GLP1R* gene from UK Biobank whole-exome sequencing data, determining their effect on structure via simulations, function via experimental evaluation of MiniGs-coupling/endocytosis, and associations with blood glucose, HbA1c, type 2 diabetes (T2D), BMI, and depressive phenotypes via association analysis with SAIGE, followed by unsupervised hierarchical clustering.

Results: We identified five distinct clusters of *GLP1R* variant structural/functional/phenotypic effects across six metabolic/mental phenotypes, determining their respective pathophysiological effects. Specifically, ClusterA 22 variants associated with improvement in metabolic/mental health. ClusterB 16 variants had positive effects on mental health, but no improvement in metabolic health. ClusterC 15 variants showed no improvement in metabolic health, potential issues with mental health. ClusterD 12 variants had positive metabolic health effects, but associated with mental health issues. Finally, ClusterF 7 variants lowered adiposity alongside glycaemic/mental health issues.

Conclusion: We report mechanistic heterogeneity of *GLP1R* variant effects on glucose metabolism, adiposity and psychological health. These mechanistic clusters suggest that individuals treated with GLP-1R mimetics respond differently to treatment, depending on their genetic composition. These insights should inform development of stratified treatment strategies.Funding: Diabetes UK 20/0006307

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P20.018.A Pharmacogenomic insights into stroke: genetic variants influencing drug metabolism, anticoagulation, and risk in Asian populations

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Background: Stroke remains a leading cause of mortality worldwide, with mitochondrial dysfunction playing a critical role in its pathogenesis. Recent findings have elucidated the relationship between pharmacogenetic variants and the increased risk of stroke, particularly in certain conditions. However, using pharmacogenetics in clinical practice to mitigate the risk of adverse drug reactions and to contribute to more efficient healthcare delivery remains underexplored. This study aims to integrate genomic data to enhance precision medicine strategies in high-risk populations and adverse-effect prevention in susceptible populations.

Material and Methods: We conducted a retrospective cohort study involving 605 stroke patients and 1493 controls from Taiwan Biobank. Participants underwent whole-genome sequencing using the Next-Generation Sequencing Analysis Acceleration Platform v20230823 and Illumina DRAGEN v3.7.5.

Results: Our analysis identified significant associations between specific pharmacogenetic variants and stroke risk, shown in Table 1. Variants include CYP3A5 (3/3), poor metabolism of statins, and anticoagulants, suggesting a strong predictive value for stroke risk in the general population; ABCG2 rs2231142 (T), increasing uric acid levels, linked to stroke risk; CYP4F2 (3), affecting vitamin K metabolism, altering warfarin response.

Conclusion: Our findings underscore the critical role of pharmacogenetic testing in the personalization of medication therapy, especially in the context of managing stroke risk. Integrating individual genetic profiles into clinical decision-making makes it possible to tailor pharmacological interventions more precisely, optimizing therapeutic efficacy. This approach ensures that drug types and dosages are specifically adapted to individual genetic dispositions, significantly enhancing treatment outcomes.

The top 15 alleles with frequencies exceeding 2% within the stroke cohort

No	Gene	Genotype	Frequency	Percentage
1	VKORC1	rs9923231 variant (T) /rs9923231 variant (T)	491	12.93
2	CYP3A5	*3/*3	314	8.27
3	SLCO1B1	*37/*37	253	6.66
4	CYP3A5	*1/*3	243	6.4
5	ABCG2	rs2231142 reference (G) /rs2231142 variant (T)	238	6.27
6	SLCO1B1	*1/*37	184	4.85
7	CYP4F2	*1/*3	144	3.79
8	DPYD	Reference/c.1627A>G (*5)	141	3.71
9	UGT1A1	*1/*6	139	3.66
10	CYP2B6	*1/*6	137	3.61
11	NUDT15	*1/*3	127	3.35
12	VKORC1	rs9923231 reference (C) /rs9923231 variant (T)	106	2.79
13	SLCO1B1	*15/*37	94	2.48
14	DPYD	c.1627A>G (*5)	85	2.24
15	IFNL3	rs12979860 reference (C) /rs12979860 variant (T)	78	2.05

C.O.I. Disclosure:
S. Tsai: None. **F.R. Tamara:** None. **S. Hsieh:** None. **J. Chang:** None.

P20.019.B Development of a novel, highly inclusive qPCR genotyping assay to detect DPYD decreased and no function variants directly from EDTA whole blood

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Background: The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD) which metabolises fluoropyrimidines to their inactive form. Individuals with decreased or no function *DPYD* alleles have an increased risk of severe adverse reactions when treated with fluoropyrimidines. The majority of available genotyping assays do not detect all Tier 1 *DPYD* variants recommended in Association of Molecular Pathology (AMP) guidelines (2024), limiting their ability to avoid drug toxicity in individuals regardless of genetic ancestry. Additionally, these assays typically require gDNA extraction prior to genotyping adding cost and time to the workflow. Herein we describe the design and development of a novel qPCR assay able to detect all Tier 1 variants in the AMP guidelines either from extracted DNA or direct from EDTA whole blood samples

Material and Methods: For each AMP Tier 1 variant two differentially labelled, allele-specific probes and a PCR primer pair were designed to uniquely amplify and provide unmatched specificity for the alleles of interest. Assay optimisation was performed with respect to sample input, mastermix formulation and cycling conditions to ensure robust performance of the assay. Performance evaluation was performed on the optimised assay workflow to establish Limit of Detection, working range, precision, and accuracy using DNA fragments, characterised *DPYD* cell line samples from Coriell, and EDTA whole blood samples.

Results: Table 1:Performance Evaluation Results

Study	Result
Limit of Detection	0.107 ng/reaction
Working Range	0.107 ng–163.84 ng/reaction
Precision	No significant impact of day, kit lot, operator or run on genotype results
Accuracy	gDNA 30/30 (100%) EDTA Whole Blood 10/10 (100%)

Conclusion: The novel DPYD qPCR genotyping assay developed demonstrates 100% accuracy and addresses current available assay limitations by providing improved inclusivity and a simplified workflow.

C.O.I. Disclosure:

A. Gasior: A. Employment (full or part-time); Significant; APIS Assay Technologies. **D. Law:** A. Employment (full or part-time); Significant; APIS Assay Technologies. **J. McDermott:** None. **W. Newman:** None. **A. Coles:** A. Employment (full or part-time); Significant; Apis Assay Technologies.

P20.020.C Optimising CYP2D6 genotyping: An inclusive digital PCR approach for star (*) allele Copy Number Variation detection

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Background: CYP2D6 metabolizes about 25% of commonly prescribed drugs, including opioids and tamoxifen. The gene encoding CYP2D6 is complex, with 163 identified star (*) alleles and frequent copy number variations (CNVs). Current clinical guidelines for CYP2D6-metabolized drugs may be biased by genetic diversity. Accurate genotyping at the *allele level is limited by the availability of CNV-quantifying assays. Existing methods often have complex workflows and lack the precision of digital PCR (dPCR). A dPCR assay to accurately determine CYP2D6 * allele CNVs could reduce health disparities by enabling pharmacogenetic testing tailored to individual genotypes. For example, oestrogen receptor-positive breast cancer patients with a poor metabolizer phenotype face higher recurrence risks with tamoxifen and may benefit from genotype-informed alternative therapies.

Material and Methods: The assay was designed to include the most relevant *CYP2D6* * alleles. Alleles with unknown or uncertain function were excluded. Alleles with an average frequency of ≥ 1.5% across biogeographical groups were prioritized, and those with ≥ 1% frequency in at least one group were considered to avoid genetic diversity bias.

Results: The assay covers 93% variants reported across diverse ethic groups, excluding alleles with uncertain functions. Despite 4% of alleles not being specifically differentiable, functional phenotypes were accurately assigned, resulting in 97% effective coverage. Proof-of-concept experiments confirmed the assay's high specificity in detecting CYP2D6 * allele CNVs.

Conclusion: This dPCR assay improves CYP2D6 * allele CNV determination, enhancing phenotypic accuracy and potentially supporting personalized treatment decisions across diverse populations.

C.O.I. Disclosure:

A. Rafaqat: A. Employment (full or part-time); Significant; Apis Assay Technologies. **A. Gasior:** A. Employment (full or part-time); Significant; Apis Assay Technologies. **J. Paveley:** A. Employment (full or part-time); Significant; Apis Assay Technologies. **A. Guardiola:** A. Employment (full or part-time); Significant; Apis Assay Technologies. **L. Fullen:** A. Employment (full or part-time); Significant; Apis Assay Technologies. **D. Law:** A. Employment (full or part-time); Significant; Apis Assay Technologies. **J. McDermott:** None. **W. Newman:** None. **A. Coles:** A. Employment (full or part-time); Significant; Apis Assay Technologies.

P20.021.D Genetics helps determine differences in response to statin treatment: analysis on short and long-term low-density lipoprotein cholesterol trajectories

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Background: understanding the genetic basis of lipid-lowering responses to statin therapy may provide valuable insights into personalized cardiovascular therapies. This study examines how genetic predisposition, as captured by polygenic scores (PGS) for low-density lipoprotein cholesterol (LDL-C), influences short and long-term changes in LDL-C levels after statin initiation.

Material and Methods: from the FinnGen cohort, we extracted 11,343 individuals with LDL-C measurements within one year before and after statin treatment initiation (short-term) and 15,864 individuals with at least five years of statin treatment and LDL-C measurements (long-term), from which we defined trajectories and disentangled independent patterns using functional principal components. We tested the effect of polygenic scores (PGS) for LDL on absolute and relative reduction of LDL (short-term) and the constant and reduction pattern (long-term) and performed genome-wide association studies.

Results: we find that individuals in the top tertile of PGS have a greater absolute reduction (8.12[6.93–9.57] mg/dl) of LDL-C in the first year but a smaller relative reduction (1.81[0.06–2.99] %) compared with the bottom tertile. While individuals with high PGS are associated with higher five-year LDL-C measurements, PGS is not associated with long-term changes in LDL-C. The GWAS detects significant genome-wide signals for relative reduction and longitudinal mean LDL-C, previously reported for LDL-C levels.

Conclusion: short-term LDL-C reduction after statin initiation appears to have a genetic basis closely linked to LDL-C regulation. Long-term changes in LDL-C levels do not appear to be regulated by genetic factors, yet individuals with high PGS for LDL have higher LDL-C in the long term.

C.O.I. Disclosure:

A. Corbetta: None. **F. Ieva:** None. **E. Di Angelantonio:** None. **A. Ganna:** None.

P20.023.B Whole Genome Sequencing (WGS): a powerful tool to gain novel insight into the *CYP2D6* gene

Stefania Zampieri[1], *Paola Tesolin*[1,2], *Giuseppe Giovanni Nardone*[2], *Alessandro Pecori*[3], *Maria Pina Concas*[1], *Mabel Troian*[1], *Aurora Santin*[1,2], *Lara Emily Rosso*[2], *Giulia Pianigiani*[1,2], *Beatrice Spedicati*[1,2], *Giorgia Girotto*[1,2]

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Background: The *CYP2D6* gene is a highly polymorphic pharmacogene involved in the metabolism of 25% of commonly used drugs. We aim to assess the feasibility of extracting relevant pharmacogenomic information from WGS data and to highlight any difference in *CYP2D6* allele frequencies between the north-eastern Italian isolated communities and the European populations.

Material and Methods: WGS was performed on 656 individuals from six different northeastern Italian genetic isolates. Cyrius software tool was used to perform *CYP2D6* genotyping and the cohort-specific allele frequencies were compared to those of a publicly available control cohort (PMID:33462347).

Results: We identified 43 haplotypes, 56% of which (24/43) were already present in the control cohort showing no significant difference in allele frequency. In contrast, 44% of haplotypes (19/43) were rare and were uniquely present in our cohort. Interestingly, 13 individuals (3 unrelated, 10 distant relatives) carried the extremely rare CYP2D6*28 × 2 allele, whose activity has not been characterized yet. Moreover, we identified a carrier of the CYP2D6*34 × 2 allele, which, to our knowledge, has never been described before.

Conclusion: The CYP2D6*28 × 2 is a rare allele which may be more frequent in the northeastern part of Italy than in the European population. However, it is not included among the most commonly tested alleles. Thus, it might explain a percentage of unsolved individual pharmacogenomic profiles. We will expand the screening to the general population to define the prevalence of the haplotypes enriched in our cohort. The findings will be relevant to tailor the pharmacological treatments in the Italian population.

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P20.024.C Precision Medicine in Action: Implementation and utility of pharmacogenomic testing in aclinical setting

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Background: Pharmacogenomics (PGx) is a rapidly developing field that utilizes an individual's genetic profile to reactively or preemptively guide drug selection, dosing adjustment, and prevent adverse drug reactions (ADRs).

Material and Methods: We conducted an IRB approved retrospective chart review study of the first 100 patients evaluated at our PGC between January 2021-November 2024.

Results: 2/3 of the patients presented to the clinic were female and 94% were referred from outpatient setting. The mean age of patients was 26.2 years old and 90% identified as White. The patients were mostly referred from genetics (48%), pediatrics (21%), and family medicine (19%). The most frequent current diagnoses were anxiety (36%), depression (30%), migraine (20%), and constipation (20%). The most common currently used medications were albuterol (27%), melatonin (23%), cetirizine (19%) and clonidine (17%), none of which have CPIC guidelines. Sertraline was the most often reported medication with ADRs (18%), followed by gabapentin, bupropion, guanfacine, and methylphenidate. The mean turnaround time was 27.6 days. All patients harbored at least one potentially actionable pharmacogenomic variant (mean: 5.6; range:1–9 per patient). The most frequently observed pharmacogenes with potentially actionable variants were: CYP2C19, CYP2D6 and CYP2C9. Preliminary analysis showed that PGx results led to patient management changes.

Conclusions: we demonstrate the feasibility and utility of PGx in a dedicated PGC. However, there are a number of challenges that need to be addressed including long turnaround times, high out-of-pocket costs, integrating PGx data into electronic health records, inclusion of underrepresented minorities, and lack of awareness of PGx testing availability.

C.O.I. Disclosure:

M. Shinawi: None. **H. Slosser:** None. **L. Peterman-Prather:** None. **J. Neidich:** None. **D. Patricia:** None. **R. Brand:** None.

P20.025.D Evidence of a protective effect of Metformin on abdominal aortic aneurysm risk: Insights from an observational study and Mendelian randomisation analysis using putative Metformin targets

Katie L. Saxby[1], *Svetlana Stoma*[1,2], *Frank Dudbridge*[3,2], *Nilesh Samani*[1,2], *Matthew J. Bown*[1,2], *Christopher P. Nelson*[1,2]

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Background: There is no proven treatment to prevent the growth of abdominal aortic aneurysm (AAA). Rupture of AAA is often fatal and surgical intervention may be offered if the risk of rupture is high. We have previously reported evidence of a potential causal association between Metformin, a drug usually prescribed to treat type 2 diabetes, and reduced AAA risk using Mendelian randomisation analysis, however the reliability of the instrument to represent Metformin treatment is uncertain. Our aim here was to assess for evidence of this association in observational data, and compare these findings with estimated causal effects.

Material and Methods: Logistic regression was used to test for evidence of association between AAA and self-reported Metformin use in 2,972 AAA cases and 89,160 propensity matched controls from UK Biobank.

Results: We found statistically significant evidence of a protective effect between Metformin treatment and AAA risk, OR 0.49 (95% CI: 0.41–0.59, $p = 3.15 \times 10^{-14}$). These findings are entirely consistent with the results of our MR analysis that found an estimated decrease in risk of 43%, OR = 0.57 (95% CI: 0.38–0.88 $p = 0.010$) per standard deviation decrease in HbA1c via Metformin gene targets, equivalent to the effect of a prescribed dosage of Metformin.

Conclusion: Our observational study found strong evidence that Metformin use reduces the risk of developing AAA, supporting our earlier findings that there is evidence of a potential causal association. Clinical trials are warranted to assess the efficacy of Metformin to reduce the risk of aneurysm growth and rupture in people with AAA.

C.O.I. Disclosure:

K.L. Saxby: None. **S. Stoma:** None. **F. Dudbridge:** None. **N. Samani:** None. **M.J. Bown:** None. **C.P. Nelson:** None.

P20.026.A *HSD3B1* as a prognostic biomarker in androgen deprivation therapy response for prostate cancer

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Background: Prostate cancer (PC) is the most prevalent neoplasm among men in Spain. Androgen deprivation therapy (ADT) is a key treatment that aims to maintain testosterone levels at castration

levels to inhibit tumor growth. However, in many patients, this treatment fails, leading to castration-resistant prostate cancer (CRPC). The objective of this study is to analyze the influence of genetic variants in the genes *HSD3B1* (rs1047303), *SRD5A2* (rs523349), *CYP17A1* (rs2486758), and *CYP19A1* (rs4775936) on the response to ADT.

Material and Methods: The rs1047303, rs523349, rs2486758, and rs4775936 variants were genotyped in 151 patients using TaqMan Genotyping technology on the QuantStudio™ 12K-Flex platform (Applied Biosystems). Clinical variables were extracted from the medical records of patients in the Andalusian Health System. The integration of experimental and clinical data was performed using SPSS Statistics software.

Results: A recessive model was established for *HSD3B1* (rs1047303), revealing that patients carrying two recessive alleles (C/C) exhibited a higher rate of therapeutic failure ($\chi^2 = 0.022$, $p = 0.027$, OR = 2.932, 95% CI = 1.132–7.593) and greater risk to developing CRPC ($\chi^2 = 0.004$, $p = 0.008$, OR = 4.349, 95% CI = 1.470–12.865). Furthermore, patients who developed CRPC were compared with those who experienced treatment efficacy. Among these groups, patients with a recessive genotype showed a higher risk of rapid progression ($\chi^2 = 0.013$, $p = 0.019$, OR = 4.219, 95% CI = 1.271–13.975). Therefore, patients carrying two alleles of rs1047303 exhibit poorer prognosis due to higher therapeutic failure rates.

Conclusion: The rs1047303 polymorphism in the *HSD3B1* gene could serve as a reliable predictive biomarker for the response to ADT and disease progression in PC patients.

C.O.I. Disclosure:

A. Torres-García: None. **B. Álvarez-González:** None. **V. Arenas-Rodríguez:** None. **F. Gutiérrez-Tejero:** None. **X. García-Villamarín:** None. **R. Morón-Romero:** None. **M. Alvarez Cubero:** None. **J. Cabeza-Barrera:** None.

P20.028.C Middle Eastern and Asian individuals possess distinct pharmacogeneticprofiles compared to Europeans

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Background: Pharmacogenetic information can be addressed during routine whole genome sequence (WGS)-based clinical genetics testing to provide personalized insights into an individual's drug metabolism, enabling more precise medication selection and dosing to minimize adverse reactions and optimize treatment efficacy.

Material and Methods: We used PharmCAT on 760 unrelated individuals (308 female) who have been enrolled in WGS clinical genetic testing and addressed tier 1 and tier 2 pharmacogenomics, which includes 17 genes and 66 drugs. The ethnic background of the individuals is from the Middle East (ME), Pakistan, Korea, and Europe. We investigated the distribution of the STAR alleles.

Results: Our results show that understudied ethnic groups (ME and Pakistan) differ significantly from Caucasian and East Asian populations in their pharmacogenetic constellation. Pakistani and Korean individuals had a significantly higher prevalence of non-functional CYP2C19 allele compared to European and ME. Importantly, about 5% of Middle Eastern and Pakistani have a deficient CYP2C9 allele, expected to result in poor metabolism of Ibuprofen. Additionally, 5.5% of ME and CE female carry a deficient G6PD allele, of whom 2 are homozygotes (associated with favism).

Conclusion: Our study, demonstrated that individuals from the Middle East and Asia have different pharmacogenomics profiles compared to Europeans. Pharmacogenetic knowledge can be impactful for certain individuals, potentially averting severe adverse reactions or guiding critical treatment decisions.

C.O.I. Disclosure:

R. Alali: A. Employment (full or part-time); Significant; Arcensus GMBH. **E. Papachristos:** A. Employment (full or part-time); Significant; Arcensus GMBH. **N. Ameziane:** A. Employment (full or part-time); Significant; Arcensus GMBH.

P20.029.D *CYP2B6* Variants: the key to a multidrug-resistant patient with paradoxical clinical response

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Background: Pharmacogenetics analyses genes encoding enzymes that regulate the metabolism of endogenous and exogenous molecules, such as the cytochrome P450 enzyme family. These enzymes are key determinants in the metabolism of many drugs, including psychotropics. Identification of inadequate metabolic activity can guide dose and drug adjustments, thereby improving treatment outcomes.

Material and Methods: A pharmacogenetic analysis was conducted in a patient with syndromic intellectual disability (ID), refractory epilepsy, and multiple adverse reactions to psychotropic medications.

Results: Thirty-year-old male patient with severe ID, disruptive behavior with severe auto and heteroagression and refratory epilepsy. Comprehensive genetic testing for an underlying etiology of the syndrome was negative. The patient presented poor therapeutic response despite being treated with multiple medications for epilepsy and aggressiveness. He also manifested severe side effects, including paradoxical reactions to antipsychotic drugs, such as haloperidol, levomepromazine, ciamemazine and aripiprazole. Pharmacogenetic analysis revealed an altered *CYP2B6* *1/*2 × 2 diplotype, categorizing the patient as an ultra-rapid metabolizer for various drug classes, including opioids, antidopaminergics, monoamine depletors, and amphetaminergic stimulants. Therapeutic adjustment with *CYP2B6*-independent drugs (such as olanzapine and quetiapine) allowed significant improvement of behavior and seizure control.

Conclusion: Pharmacogenomics insights enabled precise therapeutic adjustments, highlighting the value of these genetic tests in clinical practice to optimize treatment outcomes and improve patient quality of life.

C.O.I. Disclosure:

I.S. Nunes: None. **J.D. Da Silva:** None. **A. Soares:** None.

P20.030.A Large-scale pharmacogenetics of antidepressants using the UK Biobank drug prescription data

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Background: Epidemiological analyses show that more than 15% of the population has taken antidepressants. Despite their

common usage, choosing an effective and safe drug for a patient usually requires several attempts. Studies suggest that ~40% of the variability in response to antidepressants can be explained by genetic factors. This study aims to determine the level of association between the occurrence of pharmacogenetic variants and correlates of treatment efficacy in depression. We used whole-exome sequencing (WES) and Electronic Health Records (EHRs) available in the UK Biobank which include 75,000 individuals with a history of antidepressant medication use.

Material and Methods: We used a custom software to identify genetic variants and call star alleles from WES. Based on prescription data, we created a personalized therapy profile for each patient, detailing their individual treatment information. The occurrence of events related to the effectiveness of pharmacotherapy (change of dose, change of drug, adverse effect) were associated with pharmacogenetics variants using linear regression and Fisher's Exact Test.

Results: We show that *SLCO1B1* variants are associated with the number of changes in dose or drug in antidepressant treatment. Moreover, the increased function of *SLCO1B1* was associated with not having SSRI as the final drug in the patient's treatment regimen.

Conclusion: The results suggest that specific pharmacogenetic variants may affect the efficacy of antidepressant therapy. Understanding these relationships could help develop more personalized treatments for depression. However, further research is essential to investigate additional factors and phenotypes that may influence the effectiveness of treatment.

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P20.031.B Effects of HLA-DRA, HLA-DQA1 and IL-6 gene variations to glatiramer acetate resistance in multiple sclerosis patients

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Background: Multiple sclerosis (MS) is among the most common autoimmune disorder characterized by inflammation and degeneration affecting central nervous system (CNS). Glatiramer acetate (GA) is an immunomodulatory drugutilized for treating relapsing-remitting MS. However, considerable number of patients do not present an appropriate response to this drug. This condition is known as GA resistance. In this study, it was aimed to investigate the relationship between nucleotide variations in HLA-DRA, HLA-DQA1 and IL-6 genes with GA resistance. Additionally, the relationship of environmental factors with MS were investigated.

Material and Methods: One hundred thirty-nine MS patients were enrolled in this study. Patients were divided into two groups as non-responders (n = 58) and responders (n = 81). After DNA was isolated from peripheral blood, rs3135388 and rs3135391 variations in HLA-DRA, rs9272346 variation in HLA-DQA1, and rs1800795 and rs1900796 variations in IL-6 were analyzed by Real-Time Polymerase Chain Reaction(RT-PCR).

Results: At the end of the study, it was found that the number of females was approximately 3 times higher in responders and 4 times higher in non-responders, compared to males. When nucleotide variations and allele distributions were compared between the groups, no significant relationship was found. Similarly, no significant relationship was found between risk factors and nucleotide variations. However, in non-responders, expanded disability status scale and lesion load were found to be statistically high.

Conclusion: In conclusion by increasing the number of patients, more meaningful results can be achieved in future studies. It is believed that elucidating pharmacogenetic characteristics (the drug response-generelationship) in MS patients using GA will contribute personalized treatment strategies.

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P20.032.C Reusing sequencing data for a pharmacogenetic passport- Stakeholder interviews on implementation barriers and facilitators

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Background: Pharmacogenetics uses individuals' genetic profiles to optimize drug treatment and prevent adverse reactions. One strategy to obtain information on pharmacogenes is to reuse sequencing data to produce a pharmacogenetic passport, providing information preemptively to healthcare professionals for utilization throughout a patient's lifetime.

Aim: To explore stakeholders' perceived barriers and facilitators of implementing a pharmacogenetic passport by reusing sequencing data to extract data on pharmacogenes, in a Dutch University Medical Center.

Methods: Semi-structured interviews were conducted among 21 stakeholders. Interviews were analyzed using thematic analysis and themes were grouped under the Constellation Perspective constructs.

Results: Perceived implementation barriers included inadequate data infrastructure, limited knowledge of pharmacogenetics, lack of (visible) guidelines or protocols, unequal access, unclear division of tasks and unclear procedures, and other hospital priorities. Perceived facilitators included the ease, efficiency and affordability to obtain pharmacogenetic test results from reused sequencing data, stakeholders' positive attitudes about patient impacts of a pharmacogenetic passport, and that patient control of their own health data is provided.

Conclusion: When considering the implementation of a pharmacogenetic passport, strategies can be developed to diminish barriers and strengthen facilitators. It is important to focus on data infrastructure, (visibility of) guidelines, clear division of tasks, and pharmacogenetic education.

C.O.I. Disclosure:

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P20.033.D Leptin gene polymorphism *(LEP* 2548 G>A) as pharmacogenomics factor in Ukrainian population

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SI "V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine", Kharkiv, Ukraine.

Background: Pharmacogenomics studies the influence of genetic factors on the effectiveness of drugs. Leptin gene polymorphism is potential pharmacogenetic marker associated with type 2 diabetes (T2DM). The project aim is to define the impact of 2548 G>A *LEP* polymorphism on the effectiveness of pharmacological therapy for T2DM.

Material and Methods: Genotyping of 61 T2DM patients (M/F: 35/26, age 53.93 ± 1.20 yrs, BMI 32.68 ± 0.77 kg/m2, HbA1c 7.54 ± 0.14%) was carried out by the RFLP method. Plasma total leptine concentration, lipid profile and FFA, AST, ALT, FRAP and others parameters were determined. Patients received various hypoglycemic therapy.

Results: Comparative analysis of biochemical parameters in groups who take/did not take metformin indicates the presence of antioxidant properties in the drug (in the group receiving metformin in carriers of the GG genotype FRAP 7622.22 ± 117.23 vs 4660.41 ± 643.24 $p = 0.023$ is significantly lower), and for carriers of the A allele: hepatoprotective effect (reduction of AST U/IL 0.68 ± 0.25 vs 1.01 ± 0.32 $p = 0.02$), and the ability of the drug to improve the lipid profile (cholesterol mmol/l 5.43 ± 1.01 vs 8.17 ± 1.25 $p = 0.000024$). Metformin has certain anti-inflammatory properties in carriers of the A allele in the group receiving the drug, as there was a significant decrease in inflammatory markers such as C-reactive protein (3.32 ± 2.91 vs 10.25 ± 1.34 $p = 0,02$) and interleukin-6 (6,47 ± 4,11 vs 16,31 ± 1,16 $p = 0,04$).

Conclusion: The effectiveness of metformin therapy depends on the presence of certain leptin's gene alleles. Understanding how genetic variants affect biomarkers allows for personalization of metformin therapy for patients with T2DM in Ukrainian population.

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P20.034.A Validation of an Integrated SNP and CNV Pharmacogenomics Panel on Buccal and Blood Specimens

Jelena Feenstra[1], *Grace Wong*[2], *Cui Wen Chua*[2], *Xiaohan Xu*[3], *Shamala Letchmanan*[3], *Sing Yi Chia*[3], *Kathleen Hayashibara, Senior Scientist, Molecular Bioiogy*[1], *Say Li Kong*[3]

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Background: Pharmacogenomics (PGx) testing aims to identify genetic variations in drug metabolism enzymes and transporter genes. It is thought to be a future enabler of individualized patient care. We developed a PGx panel with integrated single nucleotide polymorphism (SNP) and copy number variation (CNV) data analytics workflow.

Material and Methods: We designed the TrueMark™ PGx SNP + CNV 64 Panel, 384-well plate (RUO) consisting of assays targeting 56 SNPs and 2 CNVs in CYP2B6, CYP2D6, CYP2C19, CYP2C9, CYP3A4, CYP3A5, CYP4F2, DPYD, NUDT15, SLCO1B1, TPMT, and VKORC1. DNA was extracted from 40 buccal swabs and 40 blood specimens collected from various ethnic groups using the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit and KingFisher™ Flex system. Extracted DNA was loaded into a 384-well qPCR plate and processed on a QuantStudio™ 12K Flex Real-Time PCR system. Genotype and CNV calls were determined using Diomni™ Design and Analysis Software 3.0. All samples were tested in parallel on the Axiom™ PangenomiX Plus Array and results were compared.

Results: Results from both buccal swabs and blood samples yielded 100% concordance for CNVs and ≥99.9% concordance for SNPs between the 2 tests. The call rate for CNVs on the TrueMark™ PGx Panel 384-well plate was ≥97.8% for buccal swabs and 100% for blood samples; while for SNPs it was ≥99.8% for buccal swabs and ≥99.9% for blood samples.

Conclusion: TrueMark™ PGx SNP + CNV 64 Panel, 384-well plate supported excellent performance with high accuracy in SNPs and CNVs detection across different sample types from various ethnic groups.

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P20.035.B Performance of a Combined SNP and Copy Number Pharmacogenomic Research Panel to Determine Phenotypes of Drug Metabolism Genes

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Background: Variations in sequence or copy number of drug metabolizing enzymes may result in altered activity towards substrates. We created the TrueMark™ PGx SNP + CNV 64 Panel, 384-well plate (RUO) containing TaqMan SNP Genotyping and Copy Number assays targeting CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP4F2, DPYD, NUDT15, SLCO1B1, TPMT, and VKORC1. Accuracy of phenotypes is presented using 167 Genetic Testing Reference Material (GeT-RM) DNAs containing targeted variants.

Material and Methods: DNAs were mixed with TaqPath™ ProAmp™ Master Mix, loaded onto pre-spotted assay plates, and run on a QuantStudio™ 7 Real-Time PCR System. Copy number and genotype results were determined by the Diomni™ Design and Analysis v3.0 software. Diplotype calls were made using AlleleTyper™ Software v1.0 with translations based on PharmGKB.

Results: CYP2D6 5'UTR and Exon 9 assays produced 100% call rate, and 100% accuracy based on consensus results from several platforms. The 64-assay panel produced calls from 99.6% of wells and produced phenotype calls consistent with consensus star alleles in 99.3 % of sample-target combinations where discrepancies were caused by alleles not covered by the panel. Diplotypes with alleles covered by the panel were identified in 100% of cases.

Conclusion: A 384-well qPCR research panel incorporating both TaqMan SNP and Copy Number assays in a single workflow suggests possibilities for development of a robust and accurate tool for determining phenotypes of key pharmacogenetic markers. The panel demonstrated high accuracy and reliability. These results underscore the potential of this panel to facilitate accurate phenotypic predictions in a streamlined workflow.

C.O.I. Disclosure:

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P21 Population Genetics and Evolutionary Genetics

P21.001.C Imputing transcriptional patterns in ancient genomes to reveal drivers of selection

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Background: Learning methodologies that leverage large-scale training data potentially allow to predict past molecular datapoints that would otherwise be lost to time. Although gene expression has been previously inferred in ancient genomes, the relatively low genotyping quality of aDNA interferes with the application of full expression predictive models. In addition, genetic drift challenges the interpretation of spatio-temporal changes in genetically-determined trait components across wide-ranging datasets.

Material and Methods: We developed gene expression and alternative splicing prediction models for 14 GTEx tissues and applied them to over a thousand high-quality, imputed ancient genomes spanning 10,000 years of Western Eurasian history. We then fitted the predicted transcriptional profiles onto multivariate models including time, space and genomic ancestry, in order to reveal gene regulatory patterns matching chronological, spatial and genetic axes of variation. Finally we explored known loci under selection in Western Eurasians and fine-mapped these selection signals onto the predicted transcriptional traits.

Results: We show improved prediction models for gene expression over those designed for non-imputed ancient genomes and introduce predicted alternative splicing in ancient samples. Among other loci, we replicated divergent predicted gene expression over time for MCM6 and SLC22A5, even when correcting for PCs and geographic coordinates, and demonstrated similar divergence in predicted alternative splicing from SLC22A5 locus.

Conclusion: Applying predictive models of genetically-determined molecular traits, like gene expression and alternative splicing patterns, to samples across a space-time transect can precisely map the molecular functions associated with selection at specific loci.

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P21.002.D Near-complete Middle Eastern genomes refine autozygosity and enhance discovery of disease-causing and population-specific variation

Younes Mokrab[1,2,3], *Mohammadmersad Ghorbani*[1], *Shabir Moosa*[1], *Zenab Siddig*[1], *Radi Farhad*[1], *Haroon Naeem*[1], *William Harvey*[4], *Francesco Kumara Mastrorosa*[4], *Katherine M. Munson*[4], *Arang Rhie*[5], *Evan E. Eichler*[4,6]

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Background: De novo genome assembly enabled by advances in long-read sequencing helped uncover previously inaccessible variation and novel sequence in the human genome. However, its impact on disease gene discovery remains largely unexplored, especially for underrepresented Middle Eastern (ME) populations, known for high consanguinity and founder mutations. Also, ME populations lack high-quality assemblies, crucial for studying genome structure, inbreeding effects, and disease risk.

Methods: We assembled near-complete phased genomes for six ME family trios($n = 18$), including healthy parents and children with unresolved neurodevelopmental conditions from Sudan, Jordan, Syria, Qatar, and Afghanistan. We analyzed structural features, local ancestry, immune receptor gene polymorphisms (*HLA/KIR*), runs of homozygosity (ROH), structural variation and novel sequences. Also, we did family trio analysis comparing assembly vs. read-based variant calling to identify deleterious variants in unresolved phenotypes. Finally, we assessed the value of population-specific references.

Results: We assembled highly accurate (Max QV = 59.3) near-complete (>99%, 14 telomere-to-telomere chromosomes; Max contig N50 = 98.2 Mb), well-phased genomes, identifying 75 novel *HLA/KIR* alleles and 42.2 Mb of novel sequence, 13.8% affecting known genes. ROH analysis revealed strong inbreeding signatures, covering one-third of Chr 6 and 12. We identified 23 de novo and recessive variants, as strong disease-causing candidates. Aligning short reads from ME samples to our assemblies improved mappability and variant calling, underscoring the importance of population-specific references.

Conclusions: Our findings underscore the significance of de novo assembly in disease variant discovery and the need for sampled ME references to detect population-relevant variation. Our study has been accepted for publication in Nature Genetics.

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P21.003.A A genetic risk variant associated with the risk of primary biliary cholangitis is inherited from Neanderthals

Chiara Caime[1], *Harold Wang*[2], *Manuela Sironi*[1], *Rosanna Asselta*[3], *Pietro Invernizzi*[1], *Sriram Sankararaman*[2], *Alessio Gerussi*[1]

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Background: Primary Biliary Cholangitis (PBC) is a rare autoimmune cholangiopathy with polygenic influences and unknown etiology. While Neanderthal-derived genetic variants have been broadly linked to autoimmune conditions, their role in PBC remains unexplored. This study investigated the potential contribution of Neanderthal-derived alleles to PBC susceptibility.

Material and Methods: We employed genetic data from four European PBC case-control cohorts (CANUK, OLD_IT, NEW_IT,

MAYO). To analyze the association between Neanderthal-derived alleles and PBC risk, we used a set of 235,592 Neanderthal Informative Markers (NIMs), incorporating genotypic and NIM principal components to adjust for population stratification. Heritability analysis focused on the CANUK cohort, estimating the contribution of NIMs to genetic variance using the RHE-mE method. Temporal dynamics of a Neanderthal-derived TNPO3 haplotype were assessed using ancient DNA data from Europe and Asia to explore allele frequency changes over time.

Results: A Neanderthal-derived variant in the TNPO3 gene (rs12531711) was significantly associated with PBC in the CANUK cohort ($p = 2.04 \times 10^{-26}$) and validated across three additional cohorts. This SNP, linked to autoimmune diseases, affects the expression of IRF5 (upregulated) and TNPO3 (downregulated) in blood. It is nearly absent in African populations but prevalent in European and Asian populations. Temporal analysis of the Neanderthal-derived haplotype revealed that its frequency increased significantly during the Bronze Age (5,000-3,000 years BP) before stabilizing. NIM analysis showed no significant contribution of these variants to PBC heritability.

Conclusion: We show that a Neanderthal-derived haplotype is associated with PBC, supporting the role of archaic variants in the susceptibility to human autoimmune diseases.

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P21.004.B The impact of selection on genotype imputation accuracy in ancient DNA studies

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Background: Ancient DNA (aDNA) provides unique insights into human evolutionary history by enabling the observation of allele frequency changes over time. However, aDNA studies face challenges due to DNA degradation and high levels of missing data. Genotype imputation using modern reference panels is a common strategy, but its accuracy declines with increasing evolutionary separation between the imputation panel and the target population, particularly in regions affected by natural selection. Selective sweeps create unique haplotype structures often poorly represented in modern panels, potentially leading to imputation artifacts. For example, imputation of Bronze Age individuals from the Pontic-Caspian Steppe inaccurately suggested high lactose tolerance frequencies at the LCT locus, later refuted by direct genotyping.

Material and Methods: We investigate biases introduced by aDNA genotype imputation in selection inference using forward-in-time simulations of human populations under various selection scenarios. We analyze imputation concordance over time and across loci experiencing selection, modeling different strengths and durations of selection.

Results: Strong selection significantly distorts imputation accuracy, particularly in regions surrounding selected loci and in samples predating the onset of selection. Strong selection leads to rapid haplotype homogenization, enhancing imputation accuracy relatively soon after selection begins. Weaker selection results in a more gradual accuracy drop, with effects persisting longer after selection starts.

Conclusion: These findings highlight the importance of considering loci selection history in aDNA imputation to avoid misleading inferences about allele frequency trajectories and the timing of adaptive events. Accounting for selection biases is crucial for accurate reconstructions of human evolutionary history.

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P21.005.C Pangenome-based inference of structurally-complex haplotypes from short-read data

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Background: Pangenome graphs model genomic variation of multiple individuals. Genotyping is inferring the alleles of a DNA sample from sequenced reads, which can be enhanced by using a pangenome graph instead of a single genome as reference.

Material and Methods: We summarise and extend cosigt, a computational workflow for genotyping structurally complex haplotypes from short-read sequencing data using a pangenome graph. For a given short-read sample and region of interest, cosigt aligns short reads to the nodes in the graph and compares the coverage of the short reads over nodes with the coverage of each diploid combination of haplotypes from the reference graph through cosine similarity, picking the combination with the highest similarity score.

Results: We benchmarked cosigt on a selection of complex loci (e.g. AMY1 and C4) using short-read individuals from the 1000G project having matching haplotypes in the HPRC(y1) pangenome graph. Comparison of the expected haplotype structure with that predicted by cosigt revealed that our method could precisely genotype ~90% of the evaluated samples, achieving ~100% at matching structurally similar haplotype clusters (~93% in a leave-one-out setting). We also applied our workflow on a diverse dataset with > 3000 individuals from 1000G, HGDP and SGDP and we found that AMY1 gene copies in the haplotypes predicted by cosigt precisely matched the copy numbers predicted by the sequenced reads with 95–99% concordance across different amylase genes.

Conclusion: Cosigt improves on prior read-based genotyping approaches by leveraging pangenome graphs to reconstruct the haplotype structure of sequenced individuals in complex loci.

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P21.006.D Curating the Portuguese variome from over 12,000 exomes: enhancing diagnostics and unlocking new research opportunities

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Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

Background: Molecular genetic testing progressed from single-gene and small multigene panels to WES and WGS, increasing variants found per case and complicating interpretation of results. Initiatives like the 1000 Genomes Project, ExAC, and gnomAD have been crucial by releasing public information on variant frequency; however, none captures specificities of the Portuguese population.

Methods: Leveraging a dataset of 12,167 patients' WES at our diagnostics laboratory, we created an anonymous reference-set, a putatively representative Portuguese cohort of 3,972 persons, iteratively resampling our dataset by municipality, and ensuring uniqueness and representativity by correcting for population biases and for geographic distribution using Census 2021 data. We focused on variants in the 81 genes in the ACMG list (v.3.2, 2023) of medically actionable secondary-findings. The ExAC open-source codebase was adapted to run locally and display that dataset.

Results: The ExAC-based browser displays allele counts and frequency of variants, by municipality, with interactive maps displaying the geographic distribution of each variant. We identified 262 PAT/L-PAT variants across 50/81 ACMG genes, among 249 individuals with actionable findings (~6.3% of our population), distributed across cardiovascular (3.1%), cancer (2.0%), miscellaneous (1.1%), and metabolic (0.1%) disorders. When displayed on the interactive map, the hemochromatosis-causing NM_000410.4(*HFE*):c.845G>A p.(Cys282Tyr) variant showed a north-south frequency-gradient corroborating previous reports.

Conclusion: We established a reference-set of common, rare, and ultra-rare genetic variants, seemingly representative of the Portuguese population. It provides a complete overview of genetic variation in the 81 actionable ACMG genes, offering valuable information for patients, practitioners, and stakeholders, aiming towards improving healthcare in Portugal.

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P21.007.A UK Biobank data shows local adaptation of immune-system genes in response to solar radiation

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Background: It is increasingly recognized that local adaption to environmental factors underlies differences in autoimmune disease prevalence across the globe. The prevalence of many autoimmune diseases increases with latitude, possibly because of vitamin D insufficiency. We hypothesise that availability of vitamin D exerts selective pressure on immune-system genes whose effects are modulated by the vitamin D receptor.

Material and Methods: We conducted a genotype-environment analysis using data from the UK Biobank on a genome-wide scale, focusing on the association with solar radiation levels to detect genomic regions that show signals of selection influenced by UV exposure. Genotyping and associated metadata, including latitude, longitude, and blood serum vitamin D levels were obtained from UK Biobank. Solar radiation levels were obtained from WorldClim. Logistic regression models were run genome-wide using Samβada for 20,000 individuals to identify variants undergoing positive selection.

Results: Genome-wide significant signals of local adaptation in response to solar radiation levels were detected at 71 loci. These loci are strongly enriched for genes involved in immune response to antigens. The strongest signal of selection, with $p = 1.35 \times 10^{-48}$, is at rs5743618, a missense SNP in *TLR1* that has previously been shown to be under selection in European populations.

Conclusion: The UK population shows strong signals of local adaptation in response to varying solar radiation levels, with immune system genes strongly enriched. Of particular interest are several genes that are involved in regulatory interactions with the vitamin D receptor, where selection may be driven by vitamin D levels.

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P21.008.B Ape genomes reveal natural protection mechanisms against human diseases

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Background: Human evolution has led to extraordinary adaptations, including a three-fold increase in brain size, the transition to upright walking, and an extended maturation and learning phase. However, these adaptations have also driven the emergence of severe disorders, such as Alzheimer's disease, bronchial asthma, and carcinomas. Here, we studied the evolutionary dynamics of disease emergence and disappearance by comparing the pathogenicity of variants in humans and other apes.

Methods: Specifically, we intersected human disease-causing variants from the ClinVar archive with high-coverage genomes of 139 great ape individuals from diverse genetic backgrounds. We next tested whether certain types of disease are more likely to undergo protection and quantified the rates at which different modes of protection evolved.

Results: We identified 342 human disease-causing variants that appear neutral in great apes. Our study revealed that the mechanisms protecting most of these variants are not restricted to a specific pathogenic variant, but are rather the result of the entire gene becoming protected in apes against pathogenic mutations. Interestingly, genes underlying diseases that affect fertility or hearing are particularly likely to evolve protection mechanisms in evolution. We further illustrated this by uncovering the compensatory mechanism in chimpanzees against a pathogenic allele in *BMP15*, which causes complete ovarian dysgenesis in humans, but is the wildtype allele in chimpanzees.

Conclusions: Overall, our targeted approach not only sheds light on the evolutionary dynamics of disease resistance but also provides a framework for leveraging these natural protection mechanisms to inform therapeutic development.

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P21.009.C Natural selection on human lipid metabolism associated with cultural and dietary shifts in eastern Holocene Europe

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Background: During the European Holocene, human populations underwent major transitions in lifestyle with important consequences for human metabolic processes. Indeed, genetic loci linked to digestion of lactase, fatty acid synthesis and antioxidant uptake have among the strongest signals of natural selection in the human genome. However, a comprehensive picture of how metabolic traits have evolved in response to dietary shifts is lacking.

Material and Methods: We combined ancient DNA data with eQTLs for blood metabolite concentrations to reconstruct metabolite profiles of Holocene Europe populations. We used cluster analyses to identify groupings of metabolites and lipid particle combinations with similar temporal trajectories, a robust statistical method to test for natural selection, and pathway analyses and isotope data from archaeological studies to interpret the results.

Results: We found strong shifts in predicted levels of VLDL, triglycerides, apolipoprotein B and glycoprotein acetyls during late Mesolithic and early Neolithic in Eastern Europe, consistent with natural selection and a shift of intake in dietary lipids from terrestrial to marine resources. A strong shift in the opposite direction was observed with the arrival of the Comb ceramic culture that brought farming and domesticated animals to the region. These predicted changes in dietary proteins are consistent with isotope data from lipid residues on ceramics from these populations.

Conclusion: We find evidence for natural selection acting to compensate for shifts in dietary intake of lipids associated with the cultural transformations of Holocene Europe. Our results support cultural and environmental factors as drivers of natural selection in human populations.

C.O.I. Disclosure:

A. Eriksson: None. **K. Kivirüüt:** None.

P21.011.A The Genome of Europe: A Reference Dataset of Genomes

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The 1 million genomes (1+MG) initiative aims to make at least 1 million whole genome sequences (WGS) accessible for use in research, health care, and prevention. Working Group 12, named Genome of Europe (GoE), aims to establish a European Reference of >500k WGS (@30x coverage) to better capture European genetic diversity. A digital Europe program (DEP)-funded initiative (also called GoE) will collect 100k samples in line with the 1+MG framework, and via the Genomics Data Infrastructure (GDI). In 2024, we introduced GoE at ESHG. We here outline the initiated GoE activities involving 51 institutes from 29 European countries. We have generated a sampling scheme for the 40 most common ancestral populations across the participating countries. We have further mapped the sequencing technologies used for this data collection and identify eligible legacy data. Three work packages establish prerequisites for GoE data collection: WP2 (Data quality & standards), WP3 (Ethical & legal) and WP4 (Data Infrastructure). WP5 will perform the data collection. WP6 will demonstrate scientific value through six use cases. WP7 handles communication, and WP1 coordinates. The GoE project has been well received, and is looking to expand, both by enabling additional countries to join, and by collaboration with other genome datasets. GoE will contribute to setting up national genome programs across Europe and assist in the implementation of genetic information in health care and prevention in a broad sense.

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P21.012.B Investigating the distribution and frequency of *CFTR* alleles in Wales using linked data

Robert T. Maddison[1], *Thomas Stoneman*[2], *Rebecca Cannings-John*[1], *Fiona Lugg-Widger*[1], *Karen R. Reed*[1], *Andrew E. Fry*[1,2]

[1]*Cardiff University, Cardiff, United Kingdom,* [2]*All-Wales Medical Genomics Service, Cardiff, United Kingdom.*

Background: Routine clinical genetic test records represent an underutilised resource for research. Allele frequencies for cystic fibrosis (CF) in Wales were previously studied in in 1993. We aimed to study the spatial distribution and change in *CFTR* allele frequencies in Wales over the last 30 years by linking routine genetic test results to data held by the Secure Anonymised Information Linkage (SAIL) Databank. SAIL is a Trusted Research Environment that provides access to anonymised health and demographic data from the population of Wales.

Methods: We imported 16,181 *CFTR* gene test reports from the All-Wales Medical Genomics Service to SAIL. The relationships between allele frequency, geographic region, deprivation, and Welsh language were studied. To avoid bias from sampling related individuals, we used linked demographic data to detect relatives. *CFTR* allele frequencies were then compared with frequencies observed in 1993.

Results: Regions in South Wales exhibited higher rates of CF births, CF carrier status, CF testing, and *CFTR* allelic diversity compared with North, Mid, and West Wales. Thirty-six different *CFTR* variants were observed, 15 of which were counted in 1993. No significant difference in allele frequencies was observed between 1993 and 2023. We estimate that around 40% of related individuals in the sample were detected.

Conclusion: Routine genetic test results can be linked to anonymised health and demographic data and used for research. *CFTR* allele frequencies in Wales have been stable over the past 30 years but exhibit regional differences. Related individuals are challenging to detect using linked anonymised demographic data.

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P21.013.C An initial version of the Korean pan-genome and structural variants using long-read sequencing

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Background: Recent genomic research has shown that a single reference genome cannot fully represent the genetic diversity of the human population, leading to reference bias and hindering the discovery of true genetic variants. This has spurred efforts to construct human pan-genomes, including the release of draft versions of the human and Chinese pan-genomes in 2023. Despite

notable genetic diversity among East Asian populations, pan-genome studies beyond China remain limited. To address this gap, we constructed the initial Korean pan-genome and identified Korean-specific structural variations.

Material and Methods: Twenty-one Koreans were sequenced using PacBio and Nanopore long-read platforms, complemented by Hi-C sequencing. High-quality telomere-to-telomere genome assemblies and annotations were generated after quality control. These assemblies were utilized to construct the Korean pan-genome using tools like Minigraph-Cactus and PanGenome-Graph Builder. The resulting pan-genome was analyzed to identify Korean-specific structural variations.

Results: We generated 28 high-quality assemblies with an average size of 3.01 Gb, an N50 of 50.34 Mb, and at least 97.5% of annotated protein-coding genes. The Korean pan-genome exceeded 3.2 Gb, revealing ~6 Mb of core genome regions absent from the current human reference genome. Structural variations specific to East Asians and Koreans were identified in the ALDH2 and APOD genes, both associated with alcohol metabolism.

Conclusion: Our Korean pan-genome is expected to uncover valuable genetic variations missing from the current reference genome, and contributes to the global effort of constructing a comprehensive human pan-genome.

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P21.014.D JaSaPaGe: Pangenome graph for Japanese and Saudi Arabian populations

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Background: Pangenomes have recently been introduced as a transformative resource for genomics, providing a more accurate understanding of genetic diversity across populations. Here we present JaSaPaGe, a pangenome graph reference for the Japanese and Saudi Arabian populations.

Material and Methods: We utilized 39.69X PacBio high-fidelity long reads, 43.68X Hi-C short reads, 44.89X Nanopore long reads, and 19.87X ultralong reads of 9 Saudi and 10 Japanese individuals to construct the diploid genome assemblies, and consequently the JaSaPaGe pangenome graph, as well as separate pangenome graphs for the Japanese and Saudi Arabian populations. To validate our pangenome graphs, we conducted short-read variant-calling for 3 Saudi samples, 3 Japanese samples, and HG001, using our graphs alongside the HPRC pangenome graph, GRCh38, and T2T-CHM13 as reference genomes.

Results: Our contiguous haplotype-phased de novo assemblies had an average N50 of 70.6 Mb and an average QV score of 64.7. Variant calling of short-read sequencing data demonstrated that using a pangenome graph resulted in detecting more variants compared to a linear reference. The performance of variant calling was comparable between our pangenome graphs and the HPRC pangenome graph. Notably, more variants were identified in Japanese and Saudi Arabian samples when their population-specific pangenomes were used as references.

Conclusion: This study shows the necessity of population-specific pangenomes. Our pangenome graphs will serve as valuable genomic resources in both Japan and Saudi Arabia.

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P21.015.A Genetic basis of human cold adaptation

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Background: Human populations at high latitudes exhibit diverse cultural and physiological adaptations, yet the role of natural selection in shaping their genetic and molecular mechanisms remains incompletely understood. While lipid metabolism and thermogenesis are known to play key roles, other biological pathways may also contribute to cold adaptation. To explore this broader genetic landscape, we conducted a genome-wide analysis to identify functional variants and pathways involved in cold stress responses in Siberian populations.

Material and Methods: We analyzed WGS data from public databases (HGDP, EGDP, SDGP) of Western, Central, and Northeast Siberian populations. We identified variants with the highest F_{ST} between Siberian and other Eurasian populations and applied a linkage disequilibrium pruning approach to refine the variant list. Each variant was annotated for empirical significance across three tests previously applied to Siberian groups in *Pagani et al*., 2016 (nSL, iHS, Tajima's D) and assessed for functional and regulatory annotations.

Results: We identified 936 independent variants uniquely common in Siberian populations, including those in pathways related to lipid metabolism, thermogenesis, and adipogenesis. Enrichment in vascular function suggests possible adaptations in vasoconstriction. Cellular signaling pathways linked to stress responses and metabolic regulation were also highlighted.

Conclusion: Our findings support the hypothesis that recent and strong natural selection acted to adapt human physiology to cold climates both directly, through thermogenesis, regulation of adipose tissue and vasoconstriction, but also indirectly as metabolic shifts in response to dietary adaptations. These results provide a foundation for future studies on human adaptation to extreme cold environments.

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A. Kolesnikova: None. **A. Eriksson:** None. **T. Kivisild:** None.

P21.016.B Mothers on the move - thousands of complete mitochondrial sequences reveal the time-layered formation of the shared maternal gene pool in Estonia and Finland

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Background: Phylogeographic reconstructions of European maternal history have mostly highlighted the expansions from glacial refugia. Here we have focused on a large-scale population-based examination of the maternal genepool of northeastern Europe, utilising complete mitochondrial sequences.

Material and Methods: We collected over 6000 full mtDNA sequences from Estonia, Finland, Sweden, Latvia and Poland with additional modern and ancient haploid genomes from published sources. We reconstructed dated phylogenetic trees for all of these sequences, established monophyletic lineages found in single populations or shared between several populations and estimated the dynamics of their effective population size.

Results: Almost 40% of Finnish individuals belong to monophyletic clusters common with linguistically related Estonians, whereas below 8% are shared with geographically close Swedish population. In Estonia, roughly 15% of individuals belong to shared Finnish-Estonian monophyletic clusters and less than 2% of Estonian individuals form haplogroups with Swedes. For the shared proportion of maternal gene pool, effective population size increases in the Bronze and Iron Age.

Conclusion: The phylogeny informed approach allows us to detect a geographic pattern previously associated primarily with male lineages. We demonstrate and discuss the importance of Bronze and Iron Age migrations on the formation of the eastern Baltic maternal gene pool by combining multiple line of evidence from modern and ancient mtDNA as well as archaeology.

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P21.017.C Genetic contribution of ancient humans in Japanese populations

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Background: Advancements in ancient genomics have revealed the ancestral admixture of modern humans. In Japan, a recent study has proposed a tripartite ancestral structure for the genetic origin of modern Japanese, involving indigenous Jomon hunter-gatherers and continental ancestors from Northeast Asia and East Asia. However, the broader implications of this ancestral structure for the genetic and phenotypic diversity of the Japanese population remain unclear.

Material and Methods: In this study, we conducted biobank-scale analyses to assess the applicability of the tripartite ancestral model and its phenotypic impact across the Japanese archipelago by integrating Biobank Japan with ancient Japanese and Eurasian genomes.

Results: We showed the suitability of the tripartite ancestral model throughout the Japanese archipelago, with significant variation in the Jomon ancestry at individual levels. This variation explained much of the genetic diversity observed in principal component analysis. Regarding phenotypic impacts, we identified a positive association between Jomon ancestry and body mass index (BMI). A genome-wide association analysis of Jomon ancestry, adjusted for geographical and ancestral substructures, identified 132 genetic markers predictive of individual Jomon ancestry. These variants are associated with significantly longer haplotypes than those from other variants, suggesting their origin from Jomon ancestry. The prediction model identified individuals who exhibit Jomon ancestry in an independent cohort of East Asian individuals from the UK Biobank and successfully replicated the positive association between Jomon ancestry and BMI.

Conclusion: Our comprehensive analysis of ancient and modern genomes provides valuable insights into the genetic contributions of ancient hunter-gatherers to contemporary populations.

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P21.018.D Expanding HLA Imputation Capabilities for the Arab Population: A 25K Reference Panel and XGBoost Based Model

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Background: The HLA region plays a critical role in disease susceptibility, immune surveillance, and allogenic transplantation compatibility. However, existing HLA imputation panels are predominantly trained on individuals of European ancestry, limiting their accuracy for underrepresented populations.

Material and Methods: To overcome this limitation, we developed the Arab HLA reference panel of 24,838 whole-genome sequenced individuals processed using Illumina DRAGEN 4.3.2, ensuring superior alignment and variant calling accuracy.

Results: We profile the HLA genetic diversity in Middle-Eastern biobank cohort, generating statistics on allele frequencies, linkage disequilibrium patterns, and population-specific haplotypes across classical HLA genes. We developed a Nextflow-based algorithm integrating three commonly used HLA genotyping tools (HISAT-genotype, HLA-HD, and T1K). All tools were standardized to use IMGT/HLA v3.49, guaranteeing uniform allele definitions and minimizing discrepancies between algorithms. This approach enabled us to derive reliable HLA alleles at both 3-field and 2-field resolution across all classical and non-classical HLA genes. We implemented an XGBoost-based model to predict HLA genotypes at 2-field resolution. The model was trained using on 80% subset of our dataset and validated on the remaining 20%. Additionally, external benchmarking was conducted using the 2,504-individual HLA reference panel from the 1000 Genomes Project. We explored HLA-disease associations for 169 traits, identifying novel and known associations with immune-mediated diseases prevalent in the Arab population.

Conclusion: Our 25K HLA reference panel and XGBoost-based imputation framework constitute the most comprehensive HLA resource for the Arab population to date, improving the accuracy of genetic analyses and advancing research in transplantation, infectious diseases, and autoimmune disorders at global scale.

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P21.019.A Effects of natural selection on the genetic architecture of core genes for type 2 diabetes identified through *trans*-effects of common variants

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Background: Waist-to-hip ratio linked to a higher risk of type 2 diabetes (T2D) and cardiovascular disease in South Asians (SA) compared to Europeans (EUR), is hypothesized to be driven by inter-population genetic differences. The "omnigenic" or sparse effector hypothesis postulates that core genes for a disease can be identified through the *trans*-effects of common SNPs. This study investigates differential selection pressure on *trans*-regulators of core genes for T2D, identified via Genome-wide Aggregated *Trans*-effects (GATE) analysis, to explain inter-population differences in T2D risk.

Material and Methods: We computed GATE scores for circulating proteins in 44,097/445,055 cases/controls (total 459105 EUR/7625 SA) of T2D in the UK Biobank using pQTLs from UKB-PPP and deCODE. We identified putative core genes through GATE scores associated with T2D ($P < 10^{-6}$). We sought evidence for differential selection in *trans*-pQTLs for these genes by calculating the fixation index (F_{st}) between EUR and SA. Deviation from neutrality in *trans*-pQTLs for core genes were ascertained via F_{st} enrichment test.

Results: *ADIPOQ*, encoding adiponectin, is the strongest of 18 discovered putative core genes for T2D ($LogOR = -0.091$, $P = 4 \times 10^{-55}$). Of these, three had large inter-ancestry differences in GATE scores: *ADIPOQ* (0.5SD), *TIMP4* (0.3SD), and *CTRB1* (0.3SD). F_{st} values were enriched in *trans*-pQTLs for *ADIPOQ* compared to neutral SNPs ($P < 10^{-10}$).

Conclusion: We provide genetic evidence that inter-population differences in *trans*-regulated *ADIPOQ* expression, inversely related to central adiposity, may mediate inter-population differences in T2D. These may occur due to differential selection on *trans*-pQTLs hinting at "thrifty genes" hypothesis.

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P21.020.B Analysis of protein-coding genetic variation in 1,043 unrelated Sardinians

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Background: Studying a population with unique genetic characteristics, such as Sardinians, offers the opportunity to discover novel functionally relevant genetic variants that have not been observed in large-scale sequencing studies and to open new avenues for understanding the impact of known disease-related variants.

Material and methods: To date, we performed the most comprehensive analysis of coding region variability in the Sardinian population. Using high-quality exome data from 1,043 unrelated individuals from a clinical genetics center in South Sardinia, we explored implications for personalized medicine. Allele frequencies were compared with those in the GnomAD v4.1 database.

Results: We identified 1,723,665 variants in canonical transcripts, with about one-third unique to Sardinians. Our analysis predicted 12,833 high-confidence and 8,855 low-confidence loss-of-function (LoF) variants, along with 2,294 splice-disrupting variants. Notably, 81.7% of LoF variants were singletons. We examined pathogenic and likely pathogenic variants enriched in Sardinians, including two known GJB2 pathogenic ones associated with genetic hearing loss, showing 61-fold and 7-fold frequency enrichment compared to Europeans; remarkably, no individuals exhibited deafness, even in a homozygous state. We also estimated the prevalence of pharmacogenetic and clinically actionable variants from the ACMG SF 3.2 gene list.

Conclusion: Our findings provide the first detailed landscape of genetic diversity in coding regions in Sardinians and offer new insights into the genetic epidemiology of monogenic diseases and pharmacogenetic traits.

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P21.021.C The first settlers of the Faroe Islands - impacts on modern heterozygosity and personalized medicine

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Background: The Faroe Islands, an isolated population of ~50,000 people, exhibit a high incidence of rare diseases, such as Primary Carnitine Deficiency (1:300). For diagnostic purposes, whole genomes from cases not affected by known mutations were sequenced at high coverage (x37). We explore the genomic variation and study the arrival and ancestry of the founding population.

Methods: We examined the relationship between the number (NROH) and sum (SROH) of ROHs to discover a bottleneck effect for ROHs larger than a minimum length. From this length and theory for the length distribution of ROHs, we estimate the timing of the initial settlement. Whole-genome SNPs and multidimensional scaling (MDS) were used to assess ancestry. SNPs within ROHs of different length intervals provided further insights into settlement timing and origins.

Results: Heterozygosity was 0.60, 0.29 and 0.93 for all, shared and unique SNPs, respectively. A population bottleneck is estimated to have begun between 50 and 300 AD, peaking around 600 AD. A distinct clustering near the central European and British populations of the 1000 Genome Project is likely the result of the population isolation and genetic drift. Further analysis using reference data including more populations from the British Isles and Scandinavia suggests the founders were Orcadians, or settlers of both Scandinavian and British ancestry.

Conclusion: The Faroese population is of European origin, possibly Orcadian or mixed central European/British/Scandinavian ancestry. This study suggests the population was founded in the 1st-4th century, potentially 2–3 centuries earlier than previously believed based on archaeological findings.

C.O.I. Disclosure:

H. Gislason: None.

P21.022.D Unraveling the impact of Neanderthal introgression on brain morphology

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Background: Present-day non-African human populations still carry about 2% of variants inherited from an ancient admixture with Neanderthal individuals. Although the biological impact of Neanderthal introgression on human complex traits has been extensively explored, a thorough analysis of its influence on traits extracted from brain structural magnetic resonance imaging (sMRI) is still lacking. Exploiting genetic associations discovered in large biobanks combining genetic and brain MRI data, we aimed to determine not only the relative impact but also the directionality of the effects of introgressed SNPs in changing brain morphology.

Material and Methods: We identified the variants tagging archaic haplotypes in UK Biobank sMRI donors, and we considered the rest of the variants as modern. We first applied RHEmc, to partition SNP heritability between archaic and modern variants. We then implemented SLDP (Signed LD Profile) regression to unravel directional effects of variants on sMRI traits, by assigning in case of Neanderthal-inherited variants a positive score to the introgressed allele and a negative score to the other allele.

Results: Our results provide evidence for the contribution of Neanderthal alleles to the genetics underlying particular areas of cerebellum, parietal and temporal lobes. We evaluate the consistency of this completely independent line of evidence with findings based on Neanderthal braincase morphological analyses.

Conclusion: Our analyses suggest that indeed the genetics underlying the morphology of these areas diverged since the Neanderthal-modern human split and that introgression-derived variants still play a role in shaping our brain morphology.

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P21.023.A Indigenous and african ancestry in the Uruguayan population

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Background: The Charrúas, an Amerindian group inhabiting Uruguay at the time of European contact, were largely eradicated as an ethnic group due to genocide. However, their cultural and genetic heritage persists in the Uruguayan population. While previous mitochondrial DNA studies confirmed Amerindian ancestry in living Uruguayans, genome-wide analyses remain scarce.

Material and Methods: We performed high-coverage whole-genome sequencing on ten Uruguayan individuals with self-declared Charrúan ancestry. Chromosomal segments of Amerindian origin were identified using ancestry deconvolution methods. Additionally, we analyzed haplotypes and compared genomic similarities between Charrúas and other indigenous groups. To better characterize Uruguay's genetic structure, we conducted low-pass whole-genome sequencing (LPS) on 850 individuals representing diverse geographic regions.

Results: We detected Amerindian ancestry in living Uruguayans, with haplotypes enriched in self-identified Charrúas but rare in other Amerindians. The genomic similarity between Charrúas and Guaraní aligns with historical records of contact. Surprisingly, we also found high similarity between Charrúas and the Diaguita from Argentina and Chile, potentially explained by Diaguita expansion and Charrúan nomadism. Preliminary results from 30 high-coverage genomes confirm Uruguay's complex admixed genetic structure.

Conclusion: This study provides the first genome-wide insights into Charrúan heritage and Uruguay's genetic diversity. While our findings confirm indigenous ancestry, larger sample sizes are needed for a complete characterization. The inclusion of 850 individuals in our LPS study will allow a more detailed understanding of Uruguay's population structure.

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P21.024.B Introducing the Y chromosome ancestral reference sequence - Improving the capture of human evolutionary information

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Background: The human Y chromosome (chrY) is widely used in evolutionary studies, but its current references represent

evolutionarily young sequences. This can lead to misleading variant calling by disregarding certain sites or calling evolutionary uninformative sites. To overcome the issue of losing evolutionary informative sites in variant calling, we created an ancestral-like reference sequence for the chrY (Y-ARS).

Material and Methods: To generate the Y-ARS, we applied maximum parsimony to determine ancestral alleles in chrY sequences of seven humans and four great ape species. We then converted all sites in the T2T-CHM13 reference to their ancestral state. To evaluate this new reference, we aligned 50 short-read sequencing samples (representing all major haplogroups) to the Y-ARS and existing references (GRCh37, GRCh38, T2T-CHM13).

Results: The alignment to the Y-ARS yielded on average the highest number of SNPs per sample (N = 691) compared to existing references (N = 498–571). Notably, when the sample and the reference were phylogenetically close (e.g., haplogroup R1b and GRCh38), the number of SNPs decreased 13-fold compared to the Y-ARS alignment. Additionally, the Y-ARS resulted in calling only evolutionary derived SNPs, while alignments to other references resulted in calling 44% ancestral SNPs due to the sites being evolutionarily derived in the reference.

Conclusion: This study demonstrates how current references fail to capture the full range of evolutionary information on the chrY. The new Y-ARS improves the capture of evolutionary derived sites, making it a valuable resource for various evolutionary genetics applications, such as TMRCA estimation, phylogenetic analyses, and demographic modeling.

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P21.025.C Following the genes - Diving deep into the genetics of Odisha's tribal populations

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Background: Endogamous indigenous populations hold key insights into the migration and evolution of modern ethnic groups. Here, we present a whole-exome sequencing of 621 self-declared healthy individuals collected from 4 districts of Odisha, a region with the highest tribal diversity in India. These individuals, collectively termed the OdiTribe belong to 13 major tribal groups representing 3 different linguistic families.

Material and Methods: We followed the GATK best practices for variant calling and ANNOVAR for annotating the variants. Quality control and statistical tests were performed using BCFtools and PLINK.

Results: We report a total of ~620k variants in our dataset. We uncovered 101,489 novel variants in our cohort, ~88% of which are OdiTribe specific (mostly singletons), ~10% are shared by other populations of India, and ~1% are shared by populations in different Asian countries. We found 220 pathogenic and likely pathogenic variants and identified pathogenic variants in the HBB (rs33915217; $p < 2.2 \times 10\text{-}16$) and HBD (rs549964658) genes with a high allele frequency for OdiTribe compared to the ExAC allele frequency. Population structure analysis by combining our exomes and GenomeAsia100k cohort captured continental and population-specific patterns of genetic variation. We found OdiTribe genetically closer to South-Asian populations, specifically the Indian tribal groups. Further, we observed 7 subclusters within the OdiTribe.

Conclusion: Overall, we reported the tribal groups of Odisha to be heterogeneous with varying degrees of homogeneity within a few groups, possibly attributed to either founder effect or population bottleneck.

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P21.026.D Unraveling the role of loss of function variants in autozygous segments: insights into gene expression and disease mechanisms

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Background: The genetic architecture of the Genes & Health UK study cohorts of Pakistani and Bangladeshi donors offers up to 100 times greater statistical power for autozygosity analyses compared to cosmopolitan populations. Long runs of homozygosity (ROH) are genomic footprints of recessive inheritance and large deleterious effects, linked to an increased prevalence of specific diseases. Loss of function (LoF) variants within autozygous segments may disrupt gene expression, potentially driving disease development.

Material and Methods: Using genetic and single-cell RNA sequencing data, we examine the effects of high autozygosity on gene expression and phenotype association. We analyse the density, localisation, and gene overlap of ROH and identified LoF SNPs within these regions. We explore the impact of LoF SNPs within ROHs on autozygosity-specific phenotypes, specifically how these contribute to genomic architecture and disease development mechanisms.

Results: Across our cohort, we identified 674,244 ROHs with a median FROH coefficient of 0.172 per donor, reflecting the high prevalence of consanguineous unions. Additionally, 9,585 LoF variants within ROH segments were found. *SKA3*, *ZAN* and *ACOT* genes are enriched for LoF variants in more than 2 cell types, being involved in mitosis, gamete recognition, and cholesterol metabolism. We aim to further explore transcriptomic effects of LoF variants in ROH using single-cell RNA sequencing data.

Conclusion: Investigating LoF variants within ROH provides a framework for understanding the genetic mechanisms underlying disease development in populations with high autozygosity rates. These findings contribute to a deeper understanding of the local genomic effects of autozygosity and its phenotypic consequences.

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P21.027.A A high-frequency Neandertal haplotype increases expression of the thermogenin Irisin in modern humans

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Background: About 47,000 years ago, modern humans encountered Neandertals while expanding into new environments,

leading to approximately 2% archaic DNA in present-day people outside Africa. Several archaic haplotypes show signs of selection that may have aided adaptation. Here, we report a Neandertal haplotype at unusually high frequency (~15% in Europe, ~24% in Southeast Asia) overlapping FNDC5, which encodes the peptide hormone Irisin. Irisin is released by skeletal muscle fibers and is key to thermogenesis.

Materials and Methods: We are currently studying this haplotype with several aims. First, we are scanning for signatures of selection which could explain its unusual high frequency. Second, we aim to identify the genetic variant causing increased FNDC5 expression by introducing the archaic variants into the modern human genome ('ancestralizing') via genome editing. Lastly, we aim to characterize the phenotypes associated with carrying this archaic haplotype.

Results: We find that variants on the Neandertal haplotype form the strongest eQTLs for FNDC5 in GTEx ($p = 6e\text{-}58$), with carriers exhibiting over double the expression compared to non-carriers. Moreover, present-day carriers show lower risk of metabolic syndrome (beta = 0.013, $p = 2e\text{-}15$) and reduced body fat (beta = 0.020, $p = 2e\text{-}9$). We also observed associations with certain cognitive phenotypes; specifically higher educational attainment and less daytime napping ($p = 5e\text{-}10$ and $2 \times e\text{-}14$, respectively), though these cognitive findings warrant caution.

Conclusion: Overall, this study illuminates how archaic gene flow helped modern humans adapt to new environments and colder climates.

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V. Soltys: None. **H. Zeberg:** None.

P21.028.B Joint effects of balancing selection and population bottlenecks on the evolution of a regulatory region of human anti-viral*APOBEC3*

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Background: Human anti-viral APOBEC3s are crucial components of the innate immune response, playing a key role in inhibiting viral replication and proliferation by inducing mutations in viral genomes. We analyzed a regulatory region of approximately 16 kb in the *APOBEC3* gene cluster, identifying three distinct haplogroups maintained at high frequencies in both African and non-African populations. Despite the long persistence of these haplogroups, one of which is shared by archaic hominins, the nucleotide diversity within each haplogroup was unusually low in non-Africans.

Material and Methods: We examined the frequency of this observation of low intra-haplogroup diversity and high inter-haplogroup divergence by coalescence simulation under neutrality and in the empirical distribution obtained through a genome scan. To explain these features and investigate the underlying evolutionary mechanisms, we performed forward simulations to model the joint effects of balancing selection and population bottlenecks.

Results: The simultaneous observations of low intra-haplogroup diversity and high inter-haplogroup divergence were incompatible with neutrality. We demonstrated the operation of balancing selection and a hitherto unexplored reduction in intra-haplogroup diversity by the forward simulations.

Conclusion: Our findings indicate that balancing selection has acted on the three haplogroups over the past ~1 million years since their emergence from the ancestral group, along with a more-than-expected reduction in intra-haplogroup diversity accompanied by the Out-of-Africa bottleneck. Most likely, the genealogy has been shaped by the combined effects of negative frequency-dependent selection and a severe reduction in effective population sizes, accompanied by the Out-of-Africa dispersal of modern humans.

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P21.031.A Understanding the DNA methylation landscape within Variable Number of Tandem Repeats (VNTRs)

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Background: Variable number of tandem repeats (VNTRs) are a significant source of genetic variation. They have been understudied as their investigation is challenging with short-read sequencing. Pacific Biosciences (PacBio) HiFi long-read sequencing technology can simultaneously sequence ~20 kb reads and estimate DNA methylation levels. We aimed to use this technology to investigate VNTRs across autosomal chromosomes, their DNA methylation levels, and the factors that may affect their methylation.

Material and Methods: PacBio HiFi sequencing (30X) was performed on blood-derived DNA from 206 individuals of European origin. Tandem Repeat Genotyping Tool (TRGT) and its repeat catalog were used to genotype VNTRs and estimate their methylation levels.

Results: There were 218,881 VNTRs on Chr1-22 in the TRGT catalog. Of these, 87,480 (40%) had variable lengths across the population, and 68,894 (31%) had at least two CpGs within their sequence in all 412 haplotypes. VNTRs tend to be methylated across the genome except for VNTRs located within 5′ upstream of the genes, 5′UTRs, and first exons overlapping CpG islands and promoter regions where the average methylation levels were typically low. The length of 2,150 (5.7%) VNTRs were associated with their methylation levels. VNTRs overlapping CTCF binding sites, open chromatin regions, TF binding sites and enhancers were more likely to be associated with their methylation levels.

Conclusion: We have been generating a comprehensive resource on VNTRs including their alleles, allele frequencies and DNA methylation levels. We have demonstrated that VNTRs are frequent genetic variations in the genome and can affect methylation contributing to human diseases.

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P21.032.B Accuracy of genetic ancestry profiles estimated by an efficient k-nearest neighbor algorithm in biobank-scale data sets

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Background: We present KANN, an efficient k-nearest neighbor regression method for individual-level genetic ancestry estimation that uses principal components (PCs). We use 18,125 samples from the Finnish Institute for Health and Welfare (THL) biobank with known reference ancestry profiles with respect to 10 Finnish source populations[1] estimated using a more computationally expensive, state-of-the-art method SOURCEFIND[2].

Material and Methods: We applied KANN with 10 PCs and compared the results to SOURCEFIND using total variation distance (TVD). We studied the marginal differences between the two methods in each ancestry source, and their distribution among samples where the methods differed highly (>0.05) for at least one source.

Results: In the test set, the mean TVD is 0.112 and, for all source populations, the lower and upper quartiles of the absolute marginal differences remain under 0.04. Among samples showing a high difference between the two methods, the difference is unevenly and asymmetrically distributed within pairs of populations, providing important information for interpretation of the estimates. For example, in samples for which KANN estimates a particularly high West Lapland (WL) component, a majority (85%) of the difference to SOURCEFIND occurs in East Lapland (EL) component, but when KANN's estimate for WL is particularly low, then only 32% of the difference occurs in EL.

Conclusion: With KANN, we can efficiently estimate ancestry profiles for biobank-scale data sets with high consistency to SOURCEFIND results. For situations where a detailed interpretation of KANN results is needed, we provide a framework for characterising uncertainty in ancestry profiles.

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P21.033.C UMI-nanopore sequencing reveals population-specific haplotype diversity in the complex lipoprotein(a) KIV-2 VNTR in a multi-ancestry sample set

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Background: Lipoprotein(a) [Lp(a)] concentrations are a major, independent risk factor for cardiovascular diseases. Concentrations are highly ancestry-specific and nearly exclusively explained by a haplotype-dependent interplay of SNPs and a coding VNTR in lipoprotein(a) *(LPA* KIV-2 repeat; up to 40 5.6 kb repeats per allele). NGS has identified several SNPs within KIV-2 but fails to resolve the complex haplotypes across VNTR units. We combined unique molecular identifiers with nanopore sequencing (UMI-ONT-Seq) to sequence the KIV-2 VNTR of 328 individuals from eleven 1000-Genomes populations.

Material and Methods: Amplicon-based UMI-ONT-Seq data was analyzed using a custom Nextflow analysis pipeline (github.com/genepi/umi-pipeline-nf) to call KIV-2 SNPs and haplotypes, infer KIV-2 copy numbers via coverage-corrected haplotypes, and describe KIV-2 haplotype diversity. Validation included plasmid mixtures and trio concordance analysis.

Results: We identified 755 SNPs across 12,453 KIV-2 repeat units, with strong agreement between UMI-ONT-Seq and NGS for variant calling ($r = 0.97$) and KIV-2 copy number estimation ($r = 0.89$). Of 10,924 observed KIV-2 haplotypes, 2,308 were unique across all populations, including common (≥200 occurrences) and ancestry-specific patterns. PCA of the haplotypes revealed ancestry-specific clusters, with African individuals showing distinct clustering and European and Asian populations displaying widespread clusters. The frequent strongly Lp(a)-lowering KIV-2 SNPs 4733G>A and 4925G>A were detected only in non-African individuals ($n = 44$). Notably, 23 of 24 unrelated 4733G>A carriers shared an identical haplotype, possibly implying recent founder effects.

Conclusion: UMI-ONT-Seq enables precise haplotyping in a highly homologous (>97%), complex VNTR. This reveals novel ancestry-specific patterns and provides insights into genetic population diversity in this dark genome region.

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P21.034.D Analysis of genetic diversity and phylogenetic relationships of D4Z4 repeats: implication for health and disease

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Background: Repetitive DNA represents about half of the human genome and plays a crucial role in physiology, disease and evolution. Notwithstanding this, the molecular function of repetitive DNA has been largely underestimated due to methodological/technical limitations. Here we use FSHD, a disease causally associated with D4Z4 repeats, as a model to investigate the features, configuration, and evolution of these repetitive sequences in *Homo sapiens* and other non-human primates.

Material and Methods: We developed an ad hoc computational workflow, to perform a comprehensive characterization of the size, number and distribution of the D4Z4 elements in the primate genome by analyzing the T2T-CHM13 assembly, 94 haplotype resolved telomere-to-telomere assemblies from the human pangenome project, and a selection of non-human primates.

Results: We identified hundreds of D4Z4 sequences in the genome with widespread inter- and intra-individual variability. Contrary to common knowledge, D4Z4 repeats localized not only at the subtelomeres of chromosomes 4q and 10q but also at the short arms of acrocentric chromosomes, interspersed with rDNA

operons, and at the pericentromeric chromatin of chromosome 1. We also reconstructed the evolutive history and the acquisition of functional elements within these repeats, that first appeared in old world monkeys spreading in the primates.

Conclusion: Our study offers an integrated approach for the study of repetitive elements and highlights the importance of telomere-to-telomere assemblies. Our results advocate for a systematic reanalysis and a critical review of previous studies on FSHD pathogenesis and diagnostics and offer new insights for the interpretation of D4Z4 elements biological role.

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P21.035.A A human-specific amino acid replacement in a brain receptor regulating food intake

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Background: The melanin-concentrating hormone receptor 2 (MCHR2) is a G protein-coupled receptor involved in energy homeostasis, stress response, and emotional regulation. A modern human-specific mutation (V324A) in MCHR2, located in the intracellular domain, distinguishes modern humans from Neanderthals, Denisovans, and all other mammals which retain the ancestral version. The functional consequences of this mutation remain unexplored.

Material and Methods: We investigated the allele frequency in large scale genomic resources including 1000 Genomes Project, gnomAD, Biobank Japan and GenomeAsia. We explored phenotypic effect using biobank data. We will conduct mammalian cell culture experiments and a BRET-based assay to compare the functional properties of the modern human (V324A) and ancestral (V324) versions of MCHR2. Given the mutation's location in the intracellular domain, we will also focus on downstream signaling pathways (e.g., cAMP, ERK, and calcium mobilization) and receptor internalization dynamics.

Results: We find the ancestral variant at frequencies approaching 1% in East Asia. Preliminary data suggest no major impact on weight or body mass index, however these phenotypic analyses are limited to mainly heterozygous carriers.

Conclusion: This project aims to uncover the functional implications of a modern human-specific MCHR2 mutation. By comparing the modern and ancestral versions, we hope to identify phenotypic consequences that may explain the selective pressures driving this mutation's fixation in modern humans.

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P21.036.B Genomic Map of Poland - Building a Reference National Database for Genetic Diversity

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Background: Many countries are currently implementing national genomic projects. Some initiatives, e.g. British 100,000 Genomes Project, or Estonian Genome Project, have already collected valuable data that can be used as references for population studies and personalized medicine. Until recently, Poland lacked such a reference. The Genomic Map of Poland (GMP) addressed this gap by providing the first comprehensive national infrastructure integrating genomic resources and bioinformatics tools to analyze genetic diversity within Poland.

Material and Methods: GMP was established by consortium of the Institute of Bioorganic Chemistry PAS and Poznan University of Technology. Anonymized samples (3970), representative of the Polish population were obtained from the biobank of the National Institute of Cardiology in Warsaw, collected as part of population health screening study. Additional biological material (saliva, blood) was collected within the project (2330). Whole genome sequencing (30–60x coverage) was performed with MGI and Illumina platforms.

Results: In total, we sequenced over 5,700 genomes of Polish residents, and generated four data subsets: ***Genetic Variability Map*** reflecting general genetic diversity within Poland (3,446 genomes representing all voivodeships, multiple counties); ***Polish Reference Genome*** assembled de novo using 10 trios and five sequencing technologies (DNBSEQ, ONT, PacBio, Hi-C and RNA-seq); ***Fen-Gen*** linking phenotype to genotype (1,004 genomes of athletes who underwent additional medical and physical examinations); ***Et-Gen*** reflecting genetic variability of five ethnic and regional minorities in Poland (1,236 genomes of Kashubians, Silesians, Lemkos, Highlanders and Poznan Bambers).

Conclusion: GMP presents the genetic landscape of Polish population, serving as a valuable resource for further research.

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P21.037.C Reference genomic database of the Czech population

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Background: Central Europe remains underrepresented in global genomic databases, limiting the interpretability of local genetic variants. The Analysis of Czech Genomes for Theranostics (A-C-G-

T) project addressed this gap by generating whole-genome sequencing (WGS) data from over 1,000 Czech adults.

Materials and Methods: Participants aged 30–55 without severe genetic diseases were enrolled if both parents originated from the same Czech region; with individuals proportionally distributed across all 14 regions of the Czech Republic. DNA libraries were prepared using PCR-free protocols and sequenced on Illumina NovaSeq 6000. Data processing followed the GATK best-practice pipeline via nf-core/sarek (v2.7.1). Technical outliers and samples with cryptic relatedness (kinship coefficient >0.0884) were removed, retaining only third-degree or more distant relatives. Population genetic analyses, including principal component analysis (PCA), were conducted to explore demographic patterns. Data were compared with global datasets, such as the 1000 Genomes Project (1KGP).

Results: The A-C-G-T database (database.acgt.cz) contains WGS data from 1,257 healthy individuals (611 females, 646 males) after removing 10 technical outliers and 23 samples with cryptic relatedness from the initial dataset (N = 1,290). In the PCA, the samples cluster with other European groups from the 1KGP, forming a distinct group that is well-separated from other clusters of European populations. An internal analysis revealed a possible genetic cline across the country.

Conclusion: This Czech-specific reference improves local variant interpretation and supports precision medicine in Central Europe. A follow-up project (A-C-G-T 2) will expand on these data by further exploring genome variation in A-C-G-T participants and analyzing patient cohorts.

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P21.038.D Genome-wide identification and analysis of great apes-specific splice sites

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Background: Alternative usage of splice sites can generate multiple transcripts from a single gene locus through the insertion or deletion of exons. Variants affecting the splicing process have been systematically identified as deleterious in some studies due to their potential impact on phenotypes. However, some variants affecting the splicing process have relatively high allele frequencies, and we speculate that such variants to be benign or even beneficial for human evolution. Genetic variants acquired during evolution can serve as a source of species-specific functions, but the role of novel alternative usage of splice sites associated with these variants remains largely unknown.

Material and Methods: We used transcriptomic data from five tissues across humans, chimpanzees, rhesus macaques and mice to identify great apes-specific splicing events and the associated variants. We also analyzed the characteristics of the identified events and their effect on the transcripts or proteins.

Results: We identified 1,043 great apes-specific splicing events including 784 annotated splicing events, 203 exon shrinkage events, 41 exon extension events, and 15 pseudo-exon activations. Through comparative analysis of the identified splice site sequences across various species, we found that most of these sites in apes tend to have higher splice site strength compared to other species. Additionally, we analyzed the impact of the identified events on transcripts and protein products, and found that approximately 41.5% of these events occur within coding sequences (CDS).

Conclusion: We consider that species-specific splice sites may be one of the important mechanisms for the acquisition of novel sequences during evolution.

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P21.039.A Challenges in finding correlations between homocysteine levels and MTHFR C677T/A1298C polymorphisms in Georgian population patients with thrombosis and pregnancy complications/infertility

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Background/Objectives: Two SNPs in the MTHFR gene, C677T and A1298C are associated with decreased enzyme activity, hyperhomocysteinemia, damage of blood vessels and thromboembolism. "Normal" values of homocysteine are a source of clinical confusion. The upper limit is around 15 µmol/L, while optimal level may be well below upper threshold. Any elevation above 5 µmol/l increases risk for cardiovascular disease and pregnancy complications. Our previous studies of 127 patients with thrombosis and 523 with pregnancy complications/infertility showed high distribution of MTHFR gene polymorphism. In this study, we aimed to analyze the correlations between homocysteine levels and MTHFR gene polymorphisms in these two groups of patients.

Methods: We chose 55 patients genotyped by multiplex PCR, with MTHFR(C677T;A1298C) polymorphisms and conducted homocysteine biochemical analysis.

Results: The table below represents our study results, which show that 8(57%) out of 14 female and 13(81.2%) out of 16 male patients with thromboembolisms had hyperhomocysteinemia. Of 25 female patients with pregnancy complications/infertility, in 15(60%) homocysteine levels were elevated, in 10(40%) in normal range.

Conclusion: Homocysteine levels were found to be more elevated in thrombotic patients than in the second group, but significant correlations between different genotypes and phenotypes were not detected. Considering that homocysteine levels are highly sensitive to blood sample handling and processing, they may fluctuate with patients' diet or preventing treatment and given the high prevalence of MTHFR gene polymorphisms in the Georgian population, we recommend including these polymorphisms in the thrombophilia diagnostic gene panel.

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Table. MTHFR C677T/A1298C polymorphisms and homocysteine level in two groups of patients.

Groups	**Thrombosis - 30 (100%) 16males (53.3%); 14females (46.7%)**					**Pregnancy complications/infertility - 25 (100%)**				
MTHFR polymorphisms	**677CT hetero**	**677TT homo**	**1298AC hetero**	**1298CC homo**	**677CT/ 1298AC compound**	**677CT hetero**	**677TT homo**	**1298AC hetero**	**1298CC homo**	**677CT/ 1298AC compound**
General distribution of polymorphisms	4 (13.3%)	2 (6.7%)	3 (10%)	7 (23.3%)	14 (46.7%)	2 (8%)	1 (4%)	12 (48%)	4 (16%)	6 (24%)
Distribution of polymorphisms in patients with hyperhomocysteinemia	2 (50%)	2 (100%)	2 (66.7%)	6 (85.7%)	9 (64.3%)	2 (100%)	1 (100%)	8 (66.7%)	1 (25%)	3 (50%)
	21 (70%); 13 males (43.3%) and 8 females (26.7%)					15 (60%); 4 (12%) with treatment; 5 (20%) without; 6 (24%) no information				
Distribution of polymorphisms in patients with normal level of homocysteine	2 (6.7%) males	0	1 (33.3%) female	1 (14.3%) female	5 (35.7%) 1 male, 4 females	0	0	4 (33.3%)	3 (75%)	3 (50%)
	9 (50%); 3 males (10%) and 6 females (20%)					10 (40%); 5 (20%) with treatment; 3 (12%) without; 2 (8%) no information				

P21.040.B Immune Cell Atlas of Ancestral and Environmental Diversity in Indonesia

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Background: Indonesia is the world's fourth most populous country, home to a diverse population of over 280 million people. The region's complex population history, ancestral components, and diverse environments present a unique immune pressure on the population. Yet, it remains consistently underrepresented in large-scale human genomic resources.

Material and Methods: We generated single-cell characterizations of peripheral blood mononuclear cells (PBMCs) from 203 individuals across Bali (representing more Island Southeast Asian-like ancestry, urban vs rural) and Papua (representing more Papuan-like ancestry, highland vs lowland) to assess how ancestral and environmental diversity impact the immune cells of Indonesians.

Results: We present preliminary results highlighting differences in cell-type proportions and cell type-specific expressions between regions (representing ancestral diversity) and villages (representing lifestyle / environment). We identified expression quantitative trait loci (eQTLs) that are both shared and specific to certain cell types, identified different patterns between regions, and also investigated overlaps with introgressed archaic haplotypes. We also discuss logistical challenges present during the project and learning points for future multisite studies.

Conclusion: We have characterized cell-type specific proportions, differential gene expression, and eQTLs within our cohort of Indonesian donors and found significant differences driven by ancestral and environmental diversities.

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P21.042.D Mapping Slovenia's Genome: The Launch of a National Genomic Reference Project

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Background: National genomic diversity is a cornerstone for advancing precision medicine. The Slovenian Reference Genome Project (SRGP), launched in January 2025, aims to construct a comprehensive pangenome representative of the Slovenian population and contribute genome data to Genome of Europe collection. By integrating cutting-edge sequencing technologies, SRGP seeks to address the limitations of current reference genomes, which fail to capture globally rare yet locally prevalent genetic variations. These insights are crucial for diagnosing rare diseases, understanding common genetic disorders, and optimizing clinical treatment.

Material and Methods: SRGP involves sequencing 500 healthy adult volunteers who consented to sequencing whole genome using long-read technology. This approach ensures the ability to produce high-resolution genomic assemblies. Advanced bioinformatics pipelines, incorporating haplotype-aware de novo assembly and methylation-based phasing, will resolve complex genomic regions. The resulting pangenome will adhere to FAIR principles,

with secure data-storage and accessibility aligned with EU 1+MG initiative.

Results: Samples of 500 volunteers and their informed consents were collected. Preliminary analyses from sequencing of 8% of the cohort demonstrate the ability of the project to provide personalized genetic insights, fostering public engagement and trust. Additionally, significant improvements in variant detection within complex genomic regions are expected to be demonstrated, enabling identification of Slovenian-specific pharmacogenomic markers and rare disease-associated variants.

Conclusion: SRGP establishes a national genomic reference critical for personalized medicine and advanced diagnostics. By resolving complex genomic regions and enabling secure, compliant data sharing, SRGP, that is also part of European initiative Genome of Europe, enhances Slovenia's contribution to international genomic initiatives.

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P21.043.A Kernel causality analysis of evolutionary amino acid changes reveals extensive nodes of coagulation factor IX variants in the development of haemophilia B

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Background: Coagulation factor IX (FIX, *F9*) is a key component of blood clotting, with mutations in FIX being associated with haemophilia B. Understanding residue changes in the evolution of FIX is essential for elucidating its structure-function relationships and the molecular basis of bleeding disorders.

Material and Methods: This study applies a kernel regression approach to perform a residue-based evolutionary analysis of FIX. Homologous sequence data were generated using Hidden Markov Model (HMMER) algorithms and were combined with functional annotations from our *F9* variant database to identify regions of evolutionary constraint and plasticity. Kernel causality analysis was utilized to estimate co-evolutionary relationships while accounting for covariates.

Results: Our findings reveal distinct patterns of conservation and epistasis. An asymmetric interdependence is suggested between the amino acid pairs Q and R. Glutamine (Q), as a kernel cause, increases arginine (R) frequencies, indicating evolutionary pathways where the introduction of glutamine compensates for the loss of arginine ($r^* = 0.1374$, $p = 0.0091$). In confirmation of this relationship, we observed nine unique R>Q variants associated with severe patient cases. The introduction of glycine (G) decreases serine (S) frequencies in homologous sequences ($r^* = -0.1383$, $p = 0.0086$), suggesting that serine substitutions derived from glycine are deleterious, a finding further supported by the *F9* variant database.

Conclusion: Our approach offers additional insights into the evolutionary forces shaping the coagulation factors and offers a framework for predicting the functional impact of genetic variations in *F9*. Our findings have broader implications for understanding protein evolution and may inform strategies for therapeutic interventions in haemophilia B.

C.O.I. Disclosure:

A. Karagöl: None. **T. Karagöl:** None. **S.J. Perkins:** None.

P21.044.B Cardiogenetics in Saguenay-Lac-Saint-Jean: deciphering the founder genetic background through a collaborative model

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Background: Saguenay-Lac-Saint-Jean (SLSJ) is a geographically isolated region in the province of Quebec, Canada. This study aims to describe the unique model of Cardiogenetics service delivery developed in SLSJ and provide evidence of the role of some probable founder alleles in the etiology of inherited cardiovascular diseases, which were previously unexplored in this population.

Material and Methods: This study was comprised of two groups: the 'affected individuals' group and the 'predictive testing group'. Patients affected with cardiomyopathies, arrhythmias, or thoracic aorta disease were referred to the Cardiogenetics Clinic between October 2014 and December 2024, and were clinically evaluated following the individual trajectory or the group genetic counseling trajectory. The predictive testing group was comprised of healthy individuals who were related by blood to individuals affected with a hereditary form of cardiovascular disease.

Results: The number of clinics performed per year constantly increased, allowing for the evaluation of 439 affected and 248 at-risk individuals. The creation of a database registry facilitated the understanding of the genotype-phenotype associations. Six genetic variants are recurrent in multiple non-consanguineous unrelated families, all of them with French-Canadian ancestry, suggesting genetic drift for these alleles in SLSJ, and enriching the understanding of the founder genetic background in this population.

Conclusion: The creation of the Cardiogenetics Clinic and the development of different evaluation trajectories have positively impacted the patients and their at-risk relatives, who benefit from personalized services, delivered in a timely manner. This experience has the potential to be replicated in other populations.

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P21.045.C Shadows of the Black Death: genetic variation in *ERAP1* and *ERAP2* selected for in response to *Yersinia pestis* infection, persist in patients with autoimmune disease

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Background: ERAP genes facilitate antigen presentation. Variation in these genes underwent selection during the plague and may now impact autoimmune disease susceptibility. This study assesses the pathogenic burden of genetic variation in the *ERAP1* and *ERAP2* genes in a cohort of patients diagnosed with Crohn's disease (CD) or ulcerative colitis (UC).

Material and Methods: This study analyses the Southampton Genetics of inflammatory bowel disease (IBD) cohort which comprises of consented patients diagnosed with IBD with rich genomics and clinical data on autoimmune comorbidities ($n_{CD} = 649$, $n_{UC} = 332$); and UKBiobank patients diagnosed with IBD and controls ($n_{CD} = 67$, $n_{UC} = 106$, $n_{control} = 4506$). In the Southampton patient-only cohort, common single variants (minor-allele-count (MAC)>0.05) were tested using logistic regression for association in UC versus CD patients. GenePy and SKAT-O were used to examine whole gene burden of likely pathogenic variants.

Results: In the Southampton IBD cohort, common variants were nominally significantly differentially distributed between UC and CD patients, but these did not withstand correction for multiple testing. Gene level analysis using all GenePy scores identified a significantly higher pathogenic burden in *ERAP1* ($p = 0.0073$) in UC compared to CD patients. No differences were identified in the Southampton cohort for *ERAP2*. Analysis of concurrent autoimmune comorbidity identified individuals with a second autoimmune diagnosis had a higher pathogenic burden in ERAP2 (vs CD $p = 0.034$(theta $= 0.457$). In UKBioBank, participants diagnosed with UC had a *higher* pathogenic burden in *ERAP1* ($p = 0.0004$), and *lower* burden in *ERAP2* ($p = 0.0006$) compared to controls.

Conclusion: Genetic variation in *ERAP1* and *ERAP2* are persistent in contemporary patient cohorts.

C.O.I. Disclosure:

L. Win: None. **S. Ennis:** None. **G. Cheng:** None.

P21.046.D Analysis of the Genetic Structure in an admixed Population from Southern Caribbean: A report from the Cartagena Cohort Study -CaReS-

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Background: Contemporary populations in Southern Caribbean are composed by a tri-hybrid admixture of European, African and Native America ancestry. Such diversity brings an opportunity to understand complex traits and human diseases; hence, descriptions of genetic structure in these populations is warrant.

Aim: Determine the genetic structure of an admixed population settled in Southern Caribbean.

Material and Methods: A cross-sectional study was conducted with adults enrolled in the Cartagena Cohort Study (CaReS). Whole blood samples were collected for genomic DNA extraction. A set of 91 Ancestry Informative Markers (AIMs) were genotyped using qPCR. A Montecarlo Markov Chain (MCMC) was performed to infer population substructure.

Results: A total of 528 adults were analyzed. A genetic structure composed by two sub-populations ($k = 2$) was found. Admixture of three ancestry groups showed a predominant contribution of European (40.0%), Native American (29.8%), and African (30.2%) features.

Conclusion: This contemporary population in Southern Caribbean showed a sub-structured genetic architecture. Further genetic association studies in this region should implement adjustments to reduce the probability of spurious associations.

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P21.047.A Assessment of the population structure of Eastern Ukraine by rs3733890 in BMHT gene and rs1801133 in MTHFR gene

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Background: Vitamin deficiency states, hyperhomocysteinemia, other metabolic features limit the use of certain drugs or cause additional prescriptions and dietary recommendations. Pharmacogenetic effects of one-carbon metabolism genes, *BMHT, MTHFR*, are known when using ademetionine, metformin hydrochloride, methotrexate, betaine hydrochloride, etc. Genetic characteristics in the world's population for these genes have wide variability. Assessment of the population structure by rs3733890 and rs1801133 is appropriate for justifying genetic testing before prescribing treatment for people with diseases of the hepatobiliary, endocrine, reproductive systems, genodermatoses, etc. in each country.

Material and Methods: Population study included people in Eastern Ukraine aged from 18 to 70 years ($n = 61$). Genotyping of SNPs was made by PCR-RFLP. An analysis of databases and projects: OMIM, Ensembl, etc. was conducted regarding rs3733890 and rs1801133.

Results: In Eastern Ukraine, the frequencies of alleles and genotypes were: for rs3733890 G:0.697, A:0.303, GG:0.443, AA:0.049, GA:0.508, for rs1801133, C:0.729, T:0.271, C/C:0.574, T/T:0.114, C/T:0.312. They do not significantly differ from the data of 1000 Genomes Project Phase 3 for Europeans: G/G:0.459, A/A:0.105, A/G:0.435 ($p = 0.979$), CC:0.406, TT:0.135, CT: 0.459 ($p = 0.942$).In all distributions, the ratio of genotypes doesn't differ from the theoretically expected equilibrium ($p > 0.001$), including the distribution of genotypes for two SNPs ($p = 0.781$). The genotypes ratio was CC/GG:0.295, CC/GA:0.262, CC/AA:0.017, CT/GG:0.115, CT/GA:0.164, CT/AA:0.033, TT/GG:0.033, TT/GA:0.082, TT/AA:0.0.

Conclusion: In Eastern Ukraine the share of genotypes for rs3733890 and rs1801133 with two or more polymorphic alleles was 44.3%. Genotyping is appropriate for clarifying the ways of correcting one-carbon metabolism, predicting the effects of therapy and its personalization.

C.O.I. Disclosure:

S. Slutu: None. **T. Borysenko:** None. **O. Fedota:** None.

P21.049.C PITX1 and TBX4 variants are rare in severe bilateral clubfoot: evidence from 162 Italian patients

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Background: Congenital clubfoot (PTC) represents one of the most common bone and joint malformations in newborns, with an incidence of 1–2 per thousand births. Although its significant impact on patients' quality of life and genetic components is well documented, with a 32.5% concordance in monozygotic twins, its

molecular basis remains largely unexplained. While previous studies have identified various transcription factors and regulators, genetic heterogeneity has complicated the identification of causative variants.

Material and Methods: We studied a cohort of 162 Italian patients with severe idiopathic bilateral clubfoot. All patients were characterized by high Pirani scores (5–6) and required Achilles tendon tenotomy. We performed genetic analysis through Sanger sequencing and SNP array, focusing on PITX1 and TBX4 genes, which are known to be involved in limb development.

Results: Our genetic analysis identified only four benign nucleotide variants in TBX4, with no significant CNVs detected in either PITX1 or TBX4. The analysis of this large independent cohort suggests that mutations in these well-characterized transcription factors play a minimal role, if any, in idiopathic clubfoot, underlining the complex genetic nature of this condition.

Conclusion: Our findings support the genetic heterogeneity of congenital clubfoot and emphasize the need for more comprehensive genetic analyses. To better understand the genetic architecture of this condition, future investigations will employ advanced techniques such as Long-Read Sequencing and Optical Genome Mapping to identify structural variants. Additionally, we plan to expand our cohort to include consanguineous families, potentially providing new insights into the genetic basis of PTC.

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P21.050.D Analysis of 1,005 human genome sequences revealed striking skewing of variant density at gene level

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Background: Genomes have regions of low or high variant density (PMID: 33741065). Whereas determination of variant density across genomes is typically performed by using a "sliding window" of arbitrary size, we focussed on determining the number of single nucleotide variants (SNVs) within all protein coding human genes across 1,000+ genomes.

Material and Methods: We examined 1,005 samples from unrelated individuals of European ancestry with different forms of congenital heart disease. 30x 150nt-paired-end genome sequencing of blood-derived gDNA. Mapping, variant calling and gene-based variant counting was accomplished with BWA, DeepVariant and VCFtools, respectively.

Results: Approximately 150 human genes show significant skewing of variant density across our cohort, i.e. these genes either have a low number of SNVs or a high number but (almost) nothing in between. Surprisingly, this skewness is confined to the gene. The flanking intergenic regions show almost the same variant density within the entire cohort. As an example of negative skewness, the CHD7 gene shows 7–52 variants in 37 samples and 198–338 variants in 961 samples. Positive skewness is seen in PTEN, where variant densitiy is dichotomous: 997 samples have <57 variants but 8 samples show 260+ SNVs. No sample has 58–259 variants. We find the same phenomenon in Tunisian and HapMap samples, but not in a dataset of 722 dog genomes (PMID: 30940804).

Prospective: Since some of the genes with skewed variant density are clinically relevant, we will search for associations of gene-specific variant density with gene expression, methylation profile, gene function and other genomic features

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P21.051.A Estimating recent population split times in non-panmictic populations

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Standard models for estimating divergence times between populations suffer from several modeling shortfalls, including an inability to distinguish between genetic drift caused by small population sizes or longer divergence times, and the reliance on an unrealistic assumption of random mating within a population. Here we present a new method for estimating recent divergence times that utilizes information in shared identity-by-descent (IBD) segments within and between populations, and which is insensitive to assumptions about random mating or population size. We apply our approach to whole-genome sequence data from Indian caste groups sampled from the Birbhum district in West Bengal (as part of the GenomeAsia 100K project), and estimate average divergence times of ~20–100 generations between different self-identified Hindu caste groups. Comparisons between Hindu caste groups and other (e.g., Austroasiatic language-speaking but geographically co-incident) groups yield slightly older divergence time estimates, and highlight the complex structure of population assimilation/replacement that highlights most of our species' history.

C.O.I. Disclosure:

J. Wall: F. Consultant/Advisory Board; Modest; Galatea Bio.

P21.052.B Population genomics reveals complex genetic history of North Asian human populations

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Background: The whole genome research on genetic diversity in human populations is accumulating significant impact for medical genetics and personalized medicine. Objective of the study is to identify population structure and reveal signals of adaptive evolution in native populations of North Asia. We also aim to demonstrate the importance of incorporating population genetic diversity and ancestry information into present biomedical research.

Material and Methods: Genome-wide analysis of genetic diversity using own WGS and SNPs array data was performed on a total sample over 2000 individuals representing 42 populations.

Results: PCA demonstrates general pattern of genetic diversity distributed according geographic locations of the populations. Major ancestral components can be distinguished as general Western- or Eastern Eurasian, and specific such as proto-Uralic, paleo-Asian, and Beringian. Uneven distribution of ROH among populations demonstrated founder effect in most North Asian populations. Analysis of genetic ancestry with ancient populations reveals significant contribution of Upper Paleolithic North Asian cultures to South and North Siberian people. Paleolithic European

hunters-gatherers and Neolithic farmers contributed to modern native South Siberian and North European populations. We demonstrate the population-specific distributions of medically relevant variants in diverse human populations.

Conclusion: Complex genetic history shaped by early migrations, isolation by distance, founder effects and natural selection has led to current genomic diversity patterns in human populations of North Asia. Future genetic studies and medical programs should take into account the genetic architecture of North Asian peoples.

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V. Stepanov: None.

P21.053.C Discovery of ARSACS evolutionary history in the Saguenay population

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Background: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is prevalent in the Saguenay-Lac-Saint-Jean (SLSJ), Quebec, Canada, due to its strong founder effect. The causal variant in the SACS gene (c.8844delT) is found in 94% of the patients. However, there might be different entries of different variants to the population. We aim to identify potential entry points and understand the evolutionary history of disease-associated haplotypes.

Material and Methods: Genealogical data was obtained for 166 patients and 207 parents (carriers), among which, we could genotype 71 patients. To perform our genealogical analysis, we used the genlib package in R. In addition, recruiting geneHapR package, we defined haplotypes of different lengths from our genotypes. Also, using Haploview software, we analyze and visualize patterns of linkage disequilibrium (LD) and construct haplotype blocks.

Results: Based on our genealogy data, we identified the couple with the greatest contribution to the carriers (parents)' gene pool. Through IBD sharing analysis, we observed a signal on chromosome 13, corresponding to the SACS gene known to cause ARSACS. We defined haplotypes of varying lengths (0.8, 2, 4, and 8 Mb) around the ARSACS variant and represented the haplotype phylogeny. The most prevalent haplotype was shared by 86% of the patients, while the remaining patients carried different types of less frequent haplotypes.

Conclusion and perspective: In summary, we identified different entries of ARSACS mutations to the Saguenay population. However, further assessment of patient haplotypes is required to confirm the time when the most recent ancestor haplotype has entered the population.

C.O.I. Disclosure:

F. Farahmand Fard: None. **C. Moreau:** None. **F. Bouchard:** None. **C. Gagnon:** None. **E. Duchesne:** None. **S. Girard:** None.

P21.054.D The Austrian Human Genome Project

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Background: Austria has been one of the last European countries without a national genome project. Based on the participation in the Genome of Europe initiative, jointly by the Medical Universities of Innsbruck, Graz, and Vienna, and the Center for Molecular Medicine in Vienna, we are now establishing a national genome infrastructure for Austria.

Material and Methods: The three main pillars of the Austrian genome project will be (a) the establishment of a national cohort of several thousand healthy individuals, (b) the integration of rare disease diagnostics at the six Centers for Medical Genetics in Austria, and (c) facilities for tumour sequencing and analysis. Sequencing will be performed at all participating centers. A state of state-of-the-art centralized computational infrastructure including data management, analysis, and interpretation pipelines will be established in collaboration with international partners.

Results: The primary outcome of the project will be disease, variant, and phenotype databases, registry facilities, a proband and patient access portal, and as well as computational services supporting interpretation, genotype imputation, and polygenic risks scores. The infrastructure will connect Austria's medical genomic centers and will be integrated into European genomic and health data networks.

Conclusion: The Austrian Human Genome Project will establish a critical genetic reference for the Austrian population, strengthen collaboration between genomic centers in Austria and Europe, and enhance healthcare and research capabilities in Austria.

C.O.I. Disclosure:

V. Beliveau: None. **M. Witsch-Baumgartner:** None. **J. Zschocke:** None.

P21.055.A Saliva: a convenient sample type enabling large-scale PacBio HiFi sequencing projects

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Background: Saliva is an attractive sample for large-scale genomic studies due to non-invasive self-collection and stability at room-temperature in DNA Genotek™ Oragene™ devices. DNA quality concerns limited its use with long-read sequencing, where high-quality, high-molecular-weight (HMW) DNA is key for optimal performance. Here, we demonstrate that high-quality PacBio® HiFi sequencing results can be obtained from saliva DNA collected into Oragene devices and extracted using Nanobind® kits manually or automatically with the ThermoFisher Kingfisher Apex system. HiFi data can be used for comprehensive variant calling and methylation analysis.

Material and Methods: HMW DNA was extracted from saliva or blood manually or automatically with Nanobind kits. HiFi WGS libraries were prepared on Hamilton NGS STAR system and sequenced using SPRQ™ chemistry on a Revio® system. HiFi reads were mapped against the human genome reference. Variant calling and methylation analysis were performed using SMRT® Link analysis pipelines.

Results: High-quality DNA was obtained from all samples. Manual and automated extraction generated comparable results. Sequencing coverage was 20x-40x per genome, sufficient for comprehensive WGS variant detection and methylation analysis. DNA from saliva samples is ~ 75%−95% human. Saliva and blood-paired samples were assessed for concordance in genome coverage quality and variant detection.

Conclusion: This method demonstrates that the extraction of DNA with Nanobind kits from saliva collected in Oragene devices is a viable alternative to blood for obtaining high-quality, long-read sequencing data. Non-invasive self-collection and ability to mail samples enable efficient, cost-effective recruitment for and

facilitate the use of long-read sequencing in large genomic studies.

C.O.I. Disclosure:

D. Moine: A. Employment (full or part-time); Significant; PacBio. **S. Kingan:** A. Employment (full or part-time); Significant; PacBio. **S. Chakraborty:** A. Employment (full or part-time); Significant; PacBio. **H. Ferrao:** A. Employment (full or part-time); Significant; PacBio. **K. Weber:** A. Employment (full or part-time); Significant; PacBio. **C. Dillane:** A. Employment (full or part-time); Significant; DNA Genotek Inc. **M. Tayeb:** A. Employment (full or part-time); Significant; DNA Genotek Inc. **D. Kilburn:** A. Employment (full or part-time); Significant; PacBio.

P22 Functional Genomics

P22.001.B Mutations in Non-Coding DNA and Type-2 Diabetes: A Pipeline for Functional Validation and Predictive Modeling

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Background: Type 2 diabetes (T2D) is associated with severe health complications. Genetic susceptibility to T2D is linked to single-nucleotide polymorphisms that overlap with cis-regulatory regions, such as enhancers, suggesting that altered transcriptional regulation contributes to this disease's susceptibility and pathogenesis. To advance our understanding of T2D, it is crucial to functionally validate these enhancers, investigate how genetic variants and spontaneous mutations impact enhancer activity, and develop predictive models for variant effects.

Material and Methods: We selected hundreds of candidate enhancers potentially involved in T2D development using data from genome-wide association studies and epigenetic features of human islets. These sequences were tested in an enhancer-reporter vector. Through FACS sorting, we isolated single cells with candidate enhancers driving GFP expression, genotyped them, and established endocrine pancreas enhancer-reporter cell lines. To investigate the functional impact of mutations on these enhancers, we applied random Cas9-mediated mutagenesis to the GFP-controlling enhancers in these reporter lines. Additionally, we created libraries of mutated enhancers through PCR-induced mutagenesis and assessed the effects of these variations using the same enhancer-reporter vector.

Results: Hundreds of sequences were validated for enhancer activity and hundreds of enhancer-reporter lines were established. Through random mutagenesis assays, we identified key nucleotides and transcription factor binding sites critical for enhancer function. Building on these findings, we developed preliminary machine learning models that predict how mutations influence enhancer activity.

Conclusion: We propose a pipeline that advances one of the most resilient frontiers in human genetics, unraveling the impact of the non-coding genome in the development of T2D.

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P22.002.C The Genomic Landscape Underlying the Segmentation Clock (Award Candidate)

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Background: Somitogenesis, the process whereby vertebrate embryos establish axial patterning, is controlled by a cycle of gene expression called the segmentation clock. Defects in the segmentation clock lead to congenital spinal abnormalities like scoliosis or spondylocostal dysostosis. Hundreds of genes that cycle with the clock have been identified. However, the regulatory elements involved in the segmentation clock have not been systematically elucidated to date.

Material and Methods: To address this, we generated diverse single cell -omics modalities (scRNA, scRNA+scATAC multiome, and scCutnTag for H3K27Ac) along a time course of the segmentation clock using a human in vitro model of somitogenesis.

Results: This enabled the identification of novel cycling genes as well as hundreds of regulatory elements that display cyclic accessibility and/or enhancer activity in the human segmentation clock. Integrating multi-modal single-cell information on chromatin occupancy (scATAC), chromatin activation state (scCutnTag), and gene expression (scRNA) revealed very good concordance between multiple regulatory layers, with some regular temporal shifts between them during each cycle. Selected new cycling regulatory elements can drive cyclic expression (using a luciferase reporter system) in our in vitro model of somitogenesis, indicating they are enhancers with cycling activity. Gene regulatory network reconstruction identified a potential negative feedback loop involving LEF1, DACT1, and DKK1 controlling cyclic antiphase expression, which has been proposed but not previously demonstrated.

Conclusion: By integrating multiple data modalities, we are able to identify which genes are cycling, the genomic elements regulating them, and the interactions between transcription factors and genes that direct cyclic gene expression.

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K. Weir: None. **M. Matsuda:** None. **X. Wang:** None. **M. Ebisuya:** None. **E. Furlong:** None.

P22.003.D Epigenetic signatures in pediatric onset Multiple Sclerosis

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Background: Pediatric-onset Multiple Sclerosis (POMS) has distinctive features, representing about 5% of all Multiple Sclerosis (MS) cases. The aim of this work is to detect epigenetic signatures potentially involved in the early onset of the disease.

Material and Methods: A multi-centric Italian cohort of 122 POMS cases and 53 pediatric healthy controls was typed with Infinium Methylation Epic v2.0 kit on whole blood. Data analysis was performed using a cross-package approach in R environment. We fitted multiple linear regression models on methylation M value matrix, correcting for gender and cell type composition. Differentially methylated probes (DMPs), genes (DMGs) and regions (DMRs) were selected according to an FDR adjusted p-value of < 0.05 and a mean absolute delta-beta (ADB) of 0.02. Gene Set Enrichment Analysis (GSEA) has been performed on the top 100 ranked DMGs.

Results: A total of 7,968 DMPs, 30 DMGs and 55 DMRs were identified. GSEA evidenced terms like "regulation of T-cell activation", "T-cell receptor binding" and "B-cell activation". Differences in methylation detected at the DMR level also indicate the contribution of genes already known in the context of Multiple Sclerosis such as BLK, BLNK, CHI3L2, CD19, CPT1B, PARP1 and LPCAT1.

Conclusion: Differences in methylation profiles between POMS and pediatric healthy subjects seem to be related to immunomodulation and immune system-specific cells. We conclude that the early onset of the disease may be mediated by the combined effect of relatively small methylation differences in crucial regulatory regions of genes involved in the immune and inflammatory response.

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P22.004.A Unraveling ribosomal DNA variation and its expression dynamics in human populations (Award Candidate)

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Background: The ribosome, composed of ribosomal proteins and ribosomal RNAs (rRNAs), is essential for protein synthesis, and its disruption has a major impact in disease. While genetic variants in ribosomal proteins are linked to diverse phenotypes, rRNAs remain underexplored due to their complex genetic architecture. Ribosomal DNA (rDNA) arrays, organized in tandem repeats, exhibit extensive intra- and inter-individual variation, with copy numbers ranging from 50 to 1000. However, studies analyzing rDNA variants have been hindered by the absence of a gold-standard reference, leading to undetected variants and false positives. Notably, rDNA copy number does not correlate with rRNA expression, as copies can be epigenetically silenced. Yet, when and which rDNA variants are silenced or differentially expressed remains unknown.

Material and Methods: Leveraging the recently completed telomere-to-telomere genome assembly, we simulated short-read sequencing data with varying rDNA copy numbers, establishing, for the first time, a gold-standard reference. Based on this data, we developed rDNAcaller, a fast and robust pipeline for accurate rDNA variant detection.

Results: Analyzing data from the 1000 Genomes Project, we characterized rDNA variation across human populations, and found population-specific silencing of rDNA variants. Additionally, using the Genotype-Tissue Expression Project, we studied rRNA expression changes between tissues and individuals of different age, sex, and BMI, revealing a major effect of aging.

Conclusion: Our study provides a comprehensive view of rDNA variation, its population-level diversity, and its dynamic expression patterns, offering new insights into its implications for human health and disease.

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P22.005.B Usmani-Riazuddin syndrome: functional characterization of novel c.196G>A, variant in the *AP1G1* gene and phenotypic insights from Zebrafish model

***Elisa Vinciarelli**[*1], Valentina Imperatore[*1], Jasmine Covarelli[1], Alessandra Mirarchi[1], Daniela Zizioli[2], Giuseppe Borsani[2], Sara Ferretti[2], Cataldo Arcuri[1], Paolo Prontera[3]*

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Background: The Adaptor Protein AP-1 is a heterotetrameric complex that plays a crucial role in intracellular trafficking and polarized localization of somatodendritic proteins in neurons. Variants in the *AP1G1* gene, encoding the gamma-1 subunit of adaptor protein complex 1 (AP1γ1), have recently been associated with Usmani-Riazuddin syndrome (USRISD MIM#619467), a very

rare genetic disorder characterized by intellectual disability (ID), speech and neurodevelopmental delay.

Material and Methods: Exome sequencing was performed in a young Italian patient with motor and language delay and a moderate form of ID. Patient's fibroblasts have been studied with immunofluorescence techniques to analyze intracellular distribution of AP-1, GM130, Clathrin, TGN46 and RAB5 (Golgi and endosomes markers). The development of the central nervous system (CNS) and locomotor behavior have been studied using zebrafish model, injecting embryos with human wild-type and mutated mRNA.

Results: We have identified a de novo heterozygous variant in *AP1G1*: c.196G>A (p.Gly66Arg), absent in the GnomAD database and predicted to be damaging. In vitro studies have demonstrated differences in subcellular localization and distribution of vesicular trafficking markers. In vivo analysis has demonstrated alterations in CNS areas (diencephalon and telencephalon), growth, movement delay, a strong phenotype of motor neurons in the trunk and a severe reduction in embryo's mobility.

Conclusion: These results suggest a critical role of the *AP1G1* subunit in regulating intracellular trafficking and CNS development, proving the pathogenicity of the c.196G>A variant. Further studies are necessary to fully understand the variant's effect on cellular functionality in order to develop a potential targeted therapeutic strategy.

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P22.006.C TAF1A ribosomopathy in a zebrafish model for juvenile-onset DCM

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Juvenile-onset dilated cardiomyopathy (DCM) is a rare genetic disorder characterized by variable phenotypic expressivity, early heart failure, and poor clinical outcomes. Bi-allelic variants in *TAF1A* (TATA-Box Binding Protein Associated Factor, RNA Polymerase I Subunit A) have been implicated as a genetic cause of juvenile-onset DCM, leading to a ribosomopathy associated with myocardial fibrosis and nucleolar segregation defects in cardiomyocytes. We previously described a 16-year-old patient with *TAF1A*-related DCM, presenting with atrial electromechanical failure and arrhythmias. To investigate the underlying disease mechanisms, we established a stable *taf1a* knockout zebrafish model using CRISPR-Cas9 genome editing. Structural characterization of myocardial tissue was performed using electron and expansion microscopy to assess high-resolution ultrastructural defects. Given the known impact of *TAF1A* deficiency on nucleolar integrity, we also examined nucleolar morphology in patient-derived fibroblasts and zebrafish cardiomyocytes. Functional assessments in zebrafish embryos included high-speed video microscopy to evaluate heart rate, fractional shortening, ventricular contractility, and arrhythmia susceptibility. Our findings provide the first functional insights into *TAF1A*-related cardiomyopathy. The *taf1a* knockout zebrafish model faithfully recapitulates key disease features and establishes a foundation for further mechanistic studies and therapeutic exploration in *TAF1A*-associated DCM.

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P22.007.D Transcriptome regulation analysis in osteoarthritis primary tissues identifies high-confidence effector genes

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Background: Osteoarthritis is a complex joint disease affecting ~600 million individuals worldwide and lacking a curative therapy. The development of disease-modifying treatments requires a comprehensive understanding of the tissue-specific effects of genetic variation. Here, we investigate gene expression regulation in macroscopically intact and degraded osteoarthritis cartilage, synovium, and fat pad tissues.

Material and Methods: We generated RNA-sequencing data from cartilage (intact: N = 263; degraded: N = 216), synovium (N = 278), and fat-pad (N = 94) tissues and matched genotype data from peripheral blood of 321 knee osteoarthritis patients undergoing joint replacement surgery. We constructed cis- and trans-eQTL maps and meta-analyzed results across tissues to find shared and tissue-specific cis-regulatory effects. We used colocalization and causal inference analyses to identify osteoarthritis effector genes.

Results: We identified 6,646 cis-eGenes in intact cartilage, 4,639 in degraded cartilage, 6,411 in synovium, and 592 in fat pad. Of 9,738 unique eGenes, 60.6% were previously unreported in osteoarthritis. We found 1,783 eGenes specific to intact cartilage, 580 to degraded cartilage, 2,308 to synovium, and 109 to fat-pad. These are regulating genes involved in chondrocyte differentiation in intact cartilage, activation of cation channels and metalloproteinases in synovium, and interferon-gamma signaling in the fat-pad. Colocalization and causal inference analyses prioritized 51 effector genes mediating osteoarthritis risk. Of these, 25 are novel osteoarthritis effector genes.

Conclusion: This study presents the largest cis-eQTL study in osteoarthritis, the first trans-eQTL study, and the first eQTL map of osteoarthritis fat pad tissue. These findings highlight tissue-specific and shared regulatory mechanisms, offering insights for advancing osteoarthritis therapeutics.

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P22.008.A Fluorescence-based CRISPR screen identifies MED12 as a new regulator of microsatellite instability

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Background: Inactivation of the DNA mismatch repair (MMR) system, due to (epi)genetic alterations of MMR genes, increases the frequency of mutations across the genome, creating a phenotype known as microsatellite instability (MSI).

Material and Methods: To detect replication slippage in cells in real time, we developed a fluorescent-reporter that becomes irreversibly activated when a slippage event occurs within a microsatellite sequence in the reporter. Subsequently, we performed CRISPR/Cas9 screens in cells carrying this reporter system to identify which genes (upon knock-out) lead to an increased rate of frameshift mutations.

Results: The activation rate of our reporter was significantly higher in MSI cell lines compared to microsatellite stable (MSS) cell lines, as expected. Additionally, KO of MMR genes MSH2 and MLH1 in MSS CRC cell line SW480 induced over 100-fold higher activation of our sensor than control genes. A genome-wide CRISPR screen in SW480 cells identified MED12 a hit. Validation studies showed that MED12 downregulates the expression of MMR genes. Consistent with this, in the TCGA colon adenocarcinoma cohort we found that MED12 mutant colon cancers that lack mutations in the known MMR genes are more likely to be of the MSI phenotype.

Conclusion: This study identified MED12 loss as a cause for MSI induction in CRC. Given that MSI is a biomarker of response to cancer immunotherapy, assessment of MED12 status might identify additional patients for immunotherapy treatment eligibility. Additional studies are needed to assess the role of MED12 in the context of Lynch syndrome, which is currently unknown.

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P22.009.B TGFB1 induced TET3 dependent regulation of OTX2 super enhancer hypomethylation promotes Group3 medulloblastoma progression

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Background: DNA methylation patterns are widely used for medulloblastoma (MB) classification, yet the functional role of enhancer methylation remains unclear.

Material and Methods: We performed bisulfite-based genome-wide sequencing on 189 MB cases, comparing 80 classified cases with 8 normal cerebellums to identify differentially methylated regions (DMRs). Gene expression data integration and validation in 109 cases identified DMR target genes. sn-ATACseq was used to assess the impact of DMRs on chromatin accessibility and tumorigenesis.

Results: A negative correlation was observed between DMR methylation at super enhancers (seDMRs) and target gene expression, particularly in Group 3 MB (G3-MB). Genes targeted by hypomethylated seDMRs were enriched in early cerebellar development pathways. OTX2-associated hypomethylated seDMRs enhanced chromatin accessibility and oncogenic activation in progenitor-like tumor cells, worsening prognosis. OTX2 binding sites within its own hypomethylated seDMRs indicated an auto-regulatory loop. Disrupting these enhancer motifs reduced OTX2 expression and attenuated tumor growth. TET3, recruited by OTX2, catalyzed active DNA demethylation, facilitating chromatin remodeling and gene activation. TET3 was upregulated by TGF-β1/Smad2 signaling. Therapeutically, nanoparticle-coated siRNA targeting TET3 effectively suppressed xenograft tumor progression.

Conclusion: Our study reveals TGF-β1/Smad-induced TET3-dependent demethylation of OTX2 super enhancers as a key driver of G3-MB progression. Targeting this pathway offers a potential therapeutic strategy.

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P22.010.C The 5000genomi@VdA project: an Italian whole genome sequencing initiative

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Background: The 5000genomi@VdA project is a unique Italian initiative funded by the Autonomous Region of Valle d'Aosta, located in the Northwest of Italy. The project is carried out by a multidisciplinary consortium led by the Italian Institute of Technology. The project aims to sequence 5,000 genomes from healthy donors and patients with neurodevelopmental, neurodegenerative, oncological, and organ transplantation diseases. Whole genome sequencing (WGS) approaches were employed to study coding and non-coding genomic regions. Through the synergistic interaction of private-public research and health care centers, the goal is to study the role of our genes in health and disease conditions and to promote the integration of WGS into the clinical domain.

Material and Methods: Genomic DNA was obtained from whole blood and, in cancer patients, tumor and non-pathological tissues. Short-read WGS was performed on the Illumina NovaSeq 6000 platform (blood: 40x coverage; tumors: 100x coverage) and, in selected cases, long-read sequencing using the PromethION device (Oxford Nanopore Technologies). Bioinformatic analysis is based on GPU-compliant and accelerated pipelines (NVIDIA Clara™ Parabricks®).

Results: We have developed the genomic and computational infrastructures to analyze and store large genomic data sets and more than 5,000 genomes have been sequenced. We also analyzed selected unsolved clinical cases with long-read sequencing as a complementary approach to accelerate research discovery and improve diagnostic rates. Additionally, genomic analysis of healthy blood donors allows characterization of the population architecture of Valle d'Aosta.

Conclusion: This study represents a strategic blueprint healthcare model for implementing WGS into clinical practice in Italy.

C.O.I. Disclosure:

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P22.011.D Investigating the role of SMCHD1 in FSHD2 and BAMS: insights from pluripotent stem cell models

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Background: SMCHD1 is a chromatin-modifying ATPase essential for epigenetic silencing during early embryonic development and regulation of long-range chromatin interactions, potentially by antagonizing CTCF binding to DNA. Germline mutations in SMCHD1 are linked to two autosomal dominant diseases: Facioscapulohumeral muscular dystrophy type 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS). FSHD2 is characterized by late-onset muscle degeneration caused by heterochromatin relaxation at the D4Z4 macrosatellite repeats due to loss-of-function (LOF) mutations, while BAMS is a rare congenital condition involving craniofacial defects arising from missense mutations in the ATPase domain of SMCHD1. Despite its clinical relevance, the functional mechanisms of SMCHD1 remain poorly understood.

Material and Methods: To elucidate SMCHD1's role, we generated SMCHD1 knockout (KO) in three human embryonic stem cell (hESC) lines using CRISPR/Cas9. These cells were characterized for DNA methylation (nanopore and bisulfite sequencing) and CTCF binding (ChIP-seq). We also introduced a BAMS-associated mutation (S135C) into hESCs and generated induced pluripotent stem cells (iPSCs) from FSHD1, FSHD2, and BAMS patients.

Results: SMCHD1 KO consistently induced ectopic CTCF binding at nearly 100 loci, including near the D4Z4 repeats, and this was independent of DNA methylation alterations. Furthermore, the BAMS-related S135C mutation did not affect CTCF binding patterns genome-wide.

Conclusion: SMCHD1 counteracts CTCF binding at specific genomic regions, including D4Z4 repeats, in a DNA methylation-independent manner. Unlike FSHD2, BAMS-related mutations do not seem to disrupt this function, suggesting distinct mechanisms underlying these diseases.

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P22.012.A A new genomic tool for assessing the functionality of thousands of SNPs simultaneously

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Background: Genome-wide association studies (GWAS) have reported thousands of risk single-nucleotide polymorphisms (SNPs) associated with refractive errors and myopia. However, understanding their functional roles remains challenging. This study introduces Barcoded Single-Nucleotide Polymorphism Sequencing (BC-SNPs-seq), a novel approach to assess the functionality of hundreds of myopia-associated SNPs simultaneously.

Material and Methods: A pilot study targeted 150 SNPs linked to the *BMP4* gene, either directly associated with myopia or in linkage disequilibrium ($r^2 \geq 0.5$) with associated SNPs. Each synthetic oligo, comprising 120 bases, included a 21-base genomic sequence centred on the SNP allele, 12 bases for a unique barcode (BC), MlyI restriction enzyme sites, and primer-annealing flanking sequences. Each SNP allele was uniquely represented by 20 barcodes, resulting in 6000 synthesized oligos. The single-stranded DNA oligos were amplified and converted to double-stranded DNA (dsDNA). These oligos were incubated with nuclear extracts (NE) from ARPE-19 retinal pigment epithelium cells or Müller cells, serving as transcription factor (TF) sources. Column separation distinguished TF-bound fragments from unbound ones, and high-throughput sequencing quantified barcode counts, representing SNP-TF interactions.

Results: BC-SNPs-seq prioritised six candidate functional SNPs that bind to the transcription factors in the ocular cell lines. Two of these SNPs showed interesting results in both cell lines, warranting further investigation.

Conclusion: BC-SNPs-seq utilises unique barcodes to efficiently screen and prioritise functional SNPs from large datasets. This innovative method significantly narrows the list of candidate SNPs for detailed functional studies, enabling deeper insights into their roles in myopia development.

C.O.I. Disclosure:

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P22.013.B Diagnostic utility of single-locus DNA methylation mark in Sotos syndrome developed by nanopore sequencing-based episignature

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Background: In various neurodevelopmental disorders (NDDs), sets of differential methylation marks (referred to as DNA methylation signatures or episignatures) are syndrome-specific and useful in evaluating the pathogenicity of detected genetic variants. These signatures have generally been tested using methylation arrays.

Methods: Nine and seven cases of molecularly diagnosed Sotos syndrome and ATR-X syndrome, respectively, were sequenced using nanopore long-read sequencing, together with 22 controls. Genome-wide differential DNA methylation analysis was performed.

Results: Long read sequencing enabled the successful extraction of two sets of differential methylation marks unique to each of Sotos syndrome and ATR-X syndrome, referred to as long-read-based DNA methylation signatures (LR-DNAm signatures), as alternatives to reported DNA methylation signatures (obtained by methylation array). Additionally, we found that a part, but not all, of the *NSD1* CpGi were hypomethylated compared with the level in controls in both cases harboring *NSD1* point mutations and those with a 5q35 submicroscopic deletion. This difference in methylation is specific to Sotos syndrome and lacking in other overgrowth intellectual disability syndromes.

Conclusions: Simultaneous evaluation of genetic and epigenetic alterations using long-read sequencing may improve the discovery of DNA methylation signatures, which may in turn increase the diagnostic yields. As an example of the outcomes of these analyses, we propose that a single-locus DNA methylation test at *NSD1* CpGi may streamline the molecular diagnosis of Sotos syndrome, regardless of the type of *NSD1* aberration.

C.O.I. Disclosure:

N. Matsumoto: None. **N. Okamoto:** None. **T. Mizuguchi:** None.

P22.014.C A landscape of X-inactivation during human T cell development

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Background: Females exhibit a more robust immune response to both self-antigens and non-self-antigens than males, resulting in a higher prevalence of autoimmune diseases but more effective responses against infection. Increased expression of X-linked immune genes in female T cells is thought to underlie this enhanced response.

Material and Methods: Here we isolate thymocytes from pediatric thymi of healthy males (46, XY), females (46, XX), a female with completely skewed X-chromosome inactivation (46, XX, cXCI) and a female with Turner syndrome (45, X0). Using whole exome sequencing, RNA sequencing and DNA methylation data, we present a sex-aware expression profile of T cell development and generate a high-resolution map of escape from X-chromosome inactivation (XCI).

Results: Unexpectedly, XCI is transcriptionally and epigenetically stable throughout T cell development, and is independent of expression of XIST, the lncRNA responsible for XCI initiation during early embryonic development. In thymocytes, several genes known to escape XCI are expressed from only one X-chromosome. Additionally, we further reveal that a second X-chromosome is dispensable for T cell development.

Conclusion: Our study thus provides a high-resolution map of XCI during human development and suggests a re-evaluation of XCI in sex differences in T cell function.

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P22.015.D Long-read sequencing analysis in Beckwith-Wiedemann syndrome caused by defects of the OCT4/SOX2 binding site

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Background: Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder with overgrowth. Hypermethylation of the *H19/IGF2*:IG-differentially methylated region (*H19*-DMR) is identified in 5% of BWS patients. The OCT4/SOX2 binding sites (OBS) involve maintaining the unmethylated *H19*-DMR. However, the precise methylation patterns in patients with OBS abnormalities remain elusive. Long-read sequencing (LRS) can detect the methylation levels separately for parental alleles. In this study, we conducted LRS to compare differences in the *H19*-DMR hypermethylation patterns between BWS patients with and without OBS abnormalities, and differences between mothers and their offspring.

Methods and Results: We have 14 sporadic BWS patients and 4 familial patients showing the *H19*-DMR hypermethylation. We performed amplicon sequence and found OBS point-mutation in 3 sporadic patients, point-mutation (chr11:2023018 C>T, hg19) in family A, and microdeletion (chr11:2022825-2025479) in family B. We performed LRS for sporadic patients with and without OBS abnormalities and all familial patients. In the patients with OBS point-mutation, the methylation levels get higher near the mutation. In the familial patients, the methylation levels are higher in offspring than in their mothers.

Assessment and Conclusion: In maternal primordial germ cells, the *H19*-DMR hypermethylation is eliminated and unmethylated after fertilization. Because the *H19*-DMR hypermethylation elimination may be insufficient, the hypermethylation levels increase with each generation. We revealed methylation patterns differences in patients with point-mutation, microdeletion, or no OBS abnormalities. Differences in the residual function of OBS may cause differences in methylation patterns.

C.O.I. Disclosure:

H. Masubuchi: None.

P22.016.A Understanding the Role of Birt-Hogg-Dubé Syndrome Associated FLCN-1 In Cilia

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Background: The Folliculin (FLCN) gene encodes a tumor suppressor protein implicated in ciliogenesis and associated with Birt-Hogg-Dubé syndrome, a ciliopathy characterized by ciliary dysfunction. FLCN plays a crucial role in mTORC1 activation and interacts with ciliary motor subunits KIF3A/KIF3B of kinesin-2 within cilia. Furthermore, FLCN has been shown to exhibit guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP) activities. However, the precise role of FLCN in cilia remains poorly understood. This study investigates the molecular mechanisms by which FLCN-1 controls the ciliary length and protein localization, providing functional insight into its contribution to ciliopathy pathogenesis.

Material and Methods: The model organism C. elegans was used to study the localization and function of FLCN-1. CRISPR/Cas9 was employed to create GFP-tagged FLCN-1 to investigate subcellular localization. Double mutants of flcn-1 with other GTPases, like RPI-2, were generated to assess genetic interactions in cilia structure and function. Quantitative fluorescence imaging measured changes in ciliary protein distribution, while FRAP experiments examined protein dynamics at the ciliary gate.

Results: Preliminary data indicate that FLCN-1 is important for maintaining cilia lengths and proper protein gating. In the flcn-1 null mutant, cilia are significantly elongated, and TRAM-1, normally excluded, aberrantly enters the cilia. Genetic interaction with RPI2 suggests a cooperative function in regulating ciliary gating and structural integrity.

Conclusion: This study provides new insight into the function flcn-1 in ciliogenesis. We now further our understanding of the molecular functions that underlie ciliopathies and thus provide a foundation for future therapeutic strategies to resolve ciliary defects.

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D. Puçak: None. **O. Kaplan:** None. **S. Cevik:** None.

P22.017.B DNA methylation in iPSC-derived cell culture models during cultivation and maturation

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Background: DNA methylation is a fundamental process in the regulation of gene expression, tailored to specific cell types and developmental stages. This study aims to investigate methylation patterns across various tissues and iPSC-derived cell culture models, providing insights into key biological processes. These findings enhance the utility of cell culture systems for studying disease mechanisms and advancing therapeutic development.

Material and Methods: We analyzed DNA methylation in samples from primary human tissues (retinae, n = 21, fibroblasts, n = 4, PBMCs, n = 7), iPSCs (n = 24), and iPSC-derived cell lines, including endothelial cells (n = 8), retinal pigment epithelium (RPE, n = 48), and retinal organoids (RO, n = 47). DNA methylation was assessed using the Human MethylationEPIC Bead Chip. To

evaluate maturation dynamics, iPSC-RPE cultures were harvested at four, ten, and 14 weeks of cultivation, while ROs were analyzed at 0.5, 1, 1.5, and 2 years.

Results: Principal component analysis revealed distinct cell type-specific clustering, while pathway enrichment analysis identified specialized pathways associated with cell type-specific differentially methylated probes (DMPs). Methylation patterns in iPSC-RPE and RO cultures evolved over time, with DMPs enriched in genes critical for differentiation and structural development. Notably, 0.5-year-old ROs differed markedly from older organoids, with aged ROs showing methylation profiles resembling those of adult retinae.

Conclusion: Our findings demonstrate that methylation patterns vary across cell types and evolve during cultivation, with RO gradually converging toward the methylation profile of mature retinae. These results underscore the importance of selecting appropriate cultivation timepoints for studying disease-related processes and highlight the dynamic nature of epigenetic regulation in iPSC-derived models.

C.O.I. Disclosure:

C. Kiel: None. **T. Strunz:** None. **B.H. Weber:** None.

P22.018.C Somatic non-allelic homologous recombination in Parkinson's disease: evidence from monozygotic twins

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Background: Parkinson's disease (PD) is a complex neurodegenerative disorder caused by the combination of genetics and environmental factors. Recent studies suggest that somatic non-allelic homologous recombination (NAHR), especially mediated by Alu and L1 elements, contributes to genomic instability in aging and neurodegeneration.

Material and methods: DNA samples from 35 pairs of monozygotic (MZ) twins discordant for PD underwent targeted sequencing to enrich for repeated elements. Somatic NAHR events were identified using the TE-reX pipeline, focusing on recombination events mediated by Alu/L1.

Results: Over 3 million recombinations were detected, primarily mediated by Alu elements. Focusing on unique somatic recombinations, we observed a significant increase in NAHR events in PD twins compared to their healthy counterparts ($P = 0.038$), particularly in Alu-mediated recombination ($P = 0.039$). Short-distance (<100 kb) recombination events were enriched in PD patients, with a significant increase in deletions ($P = 0.0035$). Additionally, Alu-mediated events in PD patients showed specific enrichment in promoter regions. Disease duration significantly influenced NAHR accumulation, especially in inter-chromosomal and long-distance (>100 kb) events ($P = 0.031$ and $P = 0.036$, respectively).

Conclusion: This study provides a global overview of the NAHR landscape in genetically identical twin pairs discordant for PD, marking the first assessment of somatic recombination in such a peculiar cohort. PD patients exhibited a distinct recombination signature, particularly in promoter regions, compared to healthy twins. Furthermore, disease duration impacted the accumulation of somatic NAHR events. In the future, we will validate these findings in sporadic PD patients, assess their specificity against other motor disorders, and investigate the role of inflammation in NAHR dysregulation.

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P22.019.D Increased signalling of the Neanderthal growth hormone receptor

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Background: Neanderthals had a robust build and a number of distinctive skeletal features. The hypothalamic-pituitary-somatotropic (HPS) axis, with growth hormone (GH) as a central signalling molecule, plays a crucial role in regulating skeletal development. To explore whether Neanderthal-specific genetic variations in the HPS axis contributed to their physical robustness, we examined genes encoding the relevant receptors and hormones.

Material and Methods: We performed genomic analysis of archaic genomes, meta-analyzed craniofacial morphology studies, used data from five biobanks (over one million individuals), and analyzed MRI scans of 220 individuals for craniofacial traits. A GH-dependent cell line was used for in vitro cell growth and signaling measurements.

Results: We identified two amino acid changes and a deletion in the Neanderthal growth hormone receptor (GHR). When expressed in a GH-dependent cell line and stimulated with pituitary GH, the Neanderthal GHR causes faster cell proliferation than the modern human GHR. We further show that some present-day humans have inherited the gene encoding the Neanderthal GHR and that these individuals are taller, exhibit more robust builds, and have craniofacial traits found in Neanderthals, such as short-rooted teeth and decreased mandibular height.

Conclusion: We show that the Neanderthal GHR responds stronger than the modern human GHR upon pituitary GH binding and that some present-day humans inherited this receptor variant from Neanderthals. As a result, they tend to have greater height, weight and muscle mass as well as some craniofacial and dental features reminiscent of Neanderthals.

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P22.020.A Cooperative insulation of regulatory domains by CTCF-dependent physical insulation and promoter competition

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The specificity of gene expression during development requires the insulation of regulatory domains to avoid inappropriate enhancer-gene interactions. In vertebrates, this insulator function is mostly attributed to clusters of CTCF sites located at topologically associating domain (TAD) boundaries. However, TAD boundaries allow some physical crosstalk across regulatory domains, which is at odds with the specific and precise expression of developmental genes. Here we show that developmental genes and nearby clusters of CTCF sites cooperatively foster the robust insulation of regulatory domains. By genetically dissecting a couple of representative loci in mouse embryonic stem cells, we show that CTCF sites prevent undesirable enhancer-gene contacts (i.e. physical insulation), while developmental genes preferentially contribute to regulatory insulation through non-structural mechanisms involving promoter competition rather than enhancer blocking. Overall, our work provides important insights into the insulation of regulatory domains, which in turn might help interpreting the pathological consequences of certain structural variants.

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P22.021.B Potential role of miR-3195 in upper airways in response to SARS-CoV-2 infection

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Background: miRNAs are non-coding RNA molecules that regulates multiples physiological processes. miRNAs play an important role in host-pathogen interactions, triggering the antiviral defence in cells or stimulate pro-inflammatory pathways. These facts have made miRNAs potential candidates for biomarkers of SARS-CoV-2 infection. The present study was carried out by novel techniques to determine the miRNA profile in the upper airways of mild COVID-19 cases to shed light on the early pathogenesis process of COVID-19.

Material and Methods: miRNAs expression from oropharyngeal/saliva samples belonging to 32 mild symptomatic COVID-19 patients and 20 age and gender-matched healthy controls was determined. miRNAs were isolated and sequenced by NGS technology. In the validation step miRNAs expression was determined by qPCR and by a new optimised ddPCR protocol for miRNAs analysis. An in-silico analysis was performed for target prediction.

Results: From the NGS analysis, miR-3195 was the most upregulated ($Log2FC = 5.23$; $p < 0.001$; $FDR < 0.001$), while miR-590-3p and miR-374a-5p were the most downregulated ($Log2FC = -4.36$ and -3.56, respectively, $p < 0.001$; $FDR < 0.05$). In the validation step, we could confirm miR-3195 expression to be increased among cases when compared to controls either through qPCR ($p = 0.03$) or through ddPCR ($p < 0.05$). In-silico analysis revealed *PFKFB4* as a target of miR-3195, which may be associated with cytokine release through the glycolytic pathway.

Conclusion: using novel techniques to analyse miRNA expression in the upper airway allowed us to identify potential biomarker candidates for COVID-19 pathogenesis. miR-3195 is suggested to participate in cytokine release regulation. Further studies in a larger cohort are needed to confirm these findings.

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P22.022.C A whole-organism landscape of X-inactivation in humans

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Background: X-chromosome inactivation (XCI) is a crucial, yet poorly understood process that results in silencing of one X-chromosome in each female cell. The stochasticity of this process typically obstructs bulk tissue analysis of X-linked allelic expression (AE). Non-mosaic females (nmXCI), in which the same parental X-chromosome is inactivated in all cells, however, are a natural and powerful genetic system to study X-inactivation in human bulk tissue.

Material and Methods: The GTEx database RNA-seq, WES and WGS data was leveraged to identify nm-XCI females based on heterozygous SNPs and AE analysis, detecting two additional nm-XCI females to the one previously known. Skewing of XCI was then determined by analysis of the AE of nonPAR X-chromosomal genes.

Results: We reveal non-mosaicism as a common feature of the human female population with implications for the penetrance and expressivity of X-linked diseases. Further, combined allele-resolution data of three nmXCI females allowed determination of XCI status across a total of 30 tissues, encompassing 380 X-linked genes, out of which 198 are characterized for the first time. Altogether this substantially increases our understanding of XCI in the human context and will be a useful reference for dissecting the genetic origin of pathologies exhibiting sex-bias.

Conclusion: Non-mosaicism in humans is a more common natural phenomenon than previously thought and can be leveraged as a powerful tool to study X-chromosomal diseases, their penetrance and genetic origin.

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P22.023.D Investigating X chromosome inactivation patterns in X-Autosome translocations using long read sequencing and the T2T genome assembly

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Background: X chromosome inactivation (XCI) is a critical mechanism in female mammals that ensures dosage compensation by silencing one of the two X chromosomes. While XCI is

typically random, genomic alterations can lead to skewed inactivation patterns. Here, we utilized long-read genome sequencing (LRS) with the PacBio Revio platform and the telomere-to-telomere (T2T) genome assembly to investigate the effects of X-autosome translocations on XCI.

Material and Methods: By combining the ability of LRS to span complex and repetitive genomic regions with the completeness of the T2T assembly, we accurately mapped translocation breakpoints. We phased the derivative and structurally healthy X chromosomes in nine carriers of X-autosome translocations. Using the epigenetic signatures from the LRS data, we (i) measured XCI, (ii) determined which X chromosome was active or inactive, and (iii) examined methylation patterns across the derivative chromosomes and translocation breakpoints.

Results: All individuals exhibited skewed XCI, indicated by methylation levels on the AR promoter (differential methylation 40–60%), confirmed by similar patterns on the RP2 promoter (differential methylation 30–70%). Preliminary analysis has shown that in two cases the derivative X is preferentially active, while in one case the structurally normal X is preferentially active. Further analysis of methylation patterns is ongoing.

Conclusion: This study highlights the power of LRS and the T2T genome in resolving structural rearrangements and their effects on epigenetic regulation. Our findings provide novel insights into the interplay between structural variants and XCI, offering a deeper understanding of their implications for female phenotypic diversity and rare disease mechanisms.

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P22.024.A Single-cell eQTL mapping identifies cell type and disease-specific genetic control of COVID-19 severity

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Background: The impact of genotype on gene expression can depend on both cellular and organismal context. The COVID-19 Multi-omics Blood ATlas (COMBAT) Consortium provided an extensive blood atlas of patients with varying COVID-19 severity, integrated with sepsis and healthy controls. However, the role of genetic regulation within gene expression in a context-specific manner remains unclear.

Methods: We identified cis-expression quantitative trait loci (cis-eQTLs) across multiple blood cell types in individuals from the COMBAT study. Using tensorQTL, we analyzed single-cell transcriptomic and genome-wide genetic data from ~400,000 cells and 76 European ancestry donors. We applied an ANOVA-based approach to detect disease-modified eQTLs and used plink to find intersecting eQTL and GWAS variants and investigate potential novel disease mechanisms.

Results: Across 15 cell types, we mapped 2,607 independent cis-eQTLs in high linkage disequilibrium (R^2>0.8) with 48 infectious and 386 inflammatory disease-associated GWAS variants, including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). Notably, we found infection-specific eQTLs absent from a general population dataset (OneK1K), such as *REL*, *IRF5* and *TRAF*, all of which were differentially regulated by infection and whose variants are associated with RA and/or IBD. We also identified infection-modified eQTLs, including *RPS26* and *ADAM10*, implying these genes may play a role in specific immune cell subsets in infection.

Conclusion: Our work demonstrates that the overriding effect of genetics on gene expression in blood immune cells is independent of infection status or severity. However, small numbers of eQTLs are modified by infection, and these differences can illustrate potentially important immune biology.

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P22.025.B Gene expression dysregulation in cohesin genes in Cornelia de Lange Syndrome patients derived cells

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Background: Cornelia de Lange Syndrome (CdLS) is a rare multisystem developmental disorder caused by variants in cohesin-related genes, coding for proteins involved in regulating chromatin structure, gene transcription, and cell cycle control. CdLS presents broad phenotypic variability, but the underlying mechanisms remain poorly understood. This study investigates gene expression profiles in lymphoblastoid cell lines (LCLs) from CdLS patients to explore potential molecular mechanisms and their possible association to clinical features.

Material and Methods: LCLs derived from two patients with *NIPBL* variants and five with *SMC1A* variants were used. RT-qPCR analyzed cohesin-related gene expression. Proliferation was assessed by Ki-67 immunofluorescence, and apoptosis rates by TUNEL assay. Phenotype severity was correlated with experimental results.

Results: Patients with *NIPBL* variants exhibited a possible compensatory mechanism between *NIPBL* and *MAU2*. In *SMC1A* patients, *SMC1A* expression remained stable, while *NIPBL*, *HDAC8*, *SMC3*, *MAU2*, and *STAG2* were dysregulated. *HDAC8* upregulation in *NIPBL* variants might enhance *SMC3* deacetylation, potentially supporting cohesin recycling. *SMC1A* variants exhibited different *HDAC8* and *SMC3* expression patterns, possibly affecting cohesin functionality. Altered *NIPBL* and *STAG2* expression appeared linked to cohesin-chromatin binding, with coordinated regulation observed in milder phenotypes. Patients with elevated apoptosis indices showed reduced proliferation rates, associated with *CCND1* downregulation. In contrast, *MYC* downregulation was observed in patients with normal apoptosis levels.

Conclusion: This study underscores the relevant role of cohesin pathway gene dysregulation in CdLS pathophysiology. The different molecular alterations in cells with variants in different cohesion components could account for phenotypic variability, and suggest the need for further studies.

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P22.026.C Establishment and study of episignatures in chromatinopathies: the specific case of Snijders Blok-Campeau syndrome

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Background: Recent advances in sequencing have enhanced patient diagnosis; however, causal mutations remain unidentified for a significant number of patients due to limited understanding of certain variants, regulatory sequences, or sequencing challenges, such as complex rearrangements. Investigating the epigenetic landscape has become essential to improve the diagnostic yield.Diseases caused by mutations in epigenetic regulators—often associated with growth abnormalities, intellectual disability, and facial dysmorphism—are prime models for studying episignatures. Among them, Snijders Blok-Campeau syndrome (ORPHA:599082), caused by pathogenic variants in the CHD3 gene, remains largely understudied.

Material and Methods: A European cohort of 30 patients, with CHD3 variants (pathogenic, likely pathogenic or uncertain significance), was analysed using the Illumina EPIC array, identifying 142 differentially methylated probes distinguishing patients from healthy controls.

Results: These regions serve as diagnostic tools for complex cases or variants of unknown significance and help uncover deregulated pathways linked to this syndrome. Comparing methylomes of patients carrying pathogenic variants in CHD3, CHD7 (CHARGE syndrome), or CHD8 (Intellectual developmental disorder with autism and macrocephaly) genes allows us to identify distinct subgroups with unique methylation profiles. This robust CHD3 episignature aids in reclassifying variants and diagnosing atypical cases.

Conclusion: Our findings advance the field of epigenetic signatures in rare diseases. We have opened new avenues for further investigation into subtypes defined by methylome assays —such as in the context of chromatinopathies—which could refine the phenotype spectrum and help predict patient outcomes.

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P22.027.D Identifying patterns of differential methylation in multiple sclerosis by positional integration approach

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Background: Over the past decade, several studies have explored the global methylation profile in multiple sclerosis (MS), revealing variable differentially methylated regions (DMRs) with limited consensus. To identify convergent DMRs, we integrated resulting data using a position-based integration of signals from these studies.

Materials and Methods: Systematic literature search identified eight methylome studies, which altogether reported 19,982 nominally significant DMRs. We used a rank sum approach, previously developed by our group, to statistically assess the results from heterogeneous sources. Obtained DMRs were mapped to tiled genomic intervals of 10 kbp. We assessed statistically significant accumulation of DMRs in given region using a permutation-based approach ($N = 1000$) and bootstrapping ($\alpha = 0.05$).

Results: Integration of methylation data revealed 172 significant regions ($p < 0.05$) with 2 regions displaying DMRs overlapping across four studies, 56 across three, and 114 across two. While we observed the most significant overlap for the well-characterised DMR in the HLA-DRB1 region, we also identified significant results in the non-coding regions. Particularly, we identified significant results in the T-cell receptor alpha chain joining gene cluster (TRAJ group) and lncRNAs regulating genes, involved in T-cell development and neuroinflammation. DMRs in proximity to protein-coding genes were enriched in pathways of immune cell regulation and differentiation, vitamin D signaling, and epigenetic modifications, highlighting the connection between environmental exposures and MS risk via epigenetic changes.

Conclusion: By integrating methylation signals across multiple MS studies, we identified consistent DMRs in both coding and non-coding genomic regions, which are associated with T-cell function, and genes regulated by MS-associated environmental factors.

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P22.028.A Human genes crucial for tolerance to metabolically induced oxidative stress - Lessons from Fanconi Anaemia

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Background: The 23 proteins of the FA/BRCA network orchestrate a precise DNA-repair process to ensure genome integrity. Fanconi Anaemia (FA) results from hereditary defects in DNA interstrand-crosslink (ICL) repair and features bone marrow failure, predisposition to acute myeloid leukaemia (AML) and head-and-neck squamous cell carcinoma (HNSCC). The sensitivity of FA cells to hyperoxia, alcohols and aldehydes indicates that metabolism of atmospheric oxygen (O_2) and alcohol is important in disease aetiology. Oxidative cellular damage is thought to drive degeneration and cancer in FA. Abrogating oxidative stress could potentially counteract degenerative processes and enhance fitness of normal FA cells.

Material and Methods: Unbiased functional-genomic CRISPR/Cas9-knockout screens under mild oxidative challenge, along with multi-omics profiling of a unique, in vitro-evolved, hyperoxia-tolerant FA-HNSCC cell line panel to find novel pathways in human cellular oxygen defence systems.

Results: CRISPR/Cas9-knockout screens in untransformed RPE1-hTERT cell models (WT vs FANCC$^{-/-}$) revealed, in addition to human orthologues of well-known yeast genes CCS, SLC31A1, SOD2, novel genes - MPV17L2, CYB5R4, and GPI - not previously linked to oxygen metabolism. CNV analysis of hyperoxia-tolerant FA-HNSCC cell lines revealed amplifications at 11q22.1–22.3 in general and 7q11.21–23, 7q22.1 unique to FA-deficient HNSCCs but not their gene-corrected counterparts. Ongoing mutation, transcriptomic and methylation analyses will yield a comprehensive understanding of genes crucial for oxygen tolerance.

Conclusion: Paraquat CRISPR-Cas9 KO screens reveal oxygen metabolism- and mitochondria-related genes. The In vitro evolved Hyperoxia-tolerant FA-C HNSCC cells are valuable for identifying genes conferring oxidative stress resistance.

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P22.029.B Comparative systems genetics of endothelial and immune cells reveals shared and distinct pathways in cardiovascular disease

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Cardiovascular disease (CVD) is an umbrella term used to describe disease of the heart and blood vessels. The endothelial lining of blood vessels is the point of contact between blood and the vasculature, and how it responds to environmental insults could determine the progression of disease. CVD has a considerable immune component, with genome wide association studies (GWAS) identifying hundreds of risk loci associated with both CVD and autoimmune disease, many of which converge on the same candidate genes.

To investigate the genetic mechanisms at CVD loci we generated a new molecular quantitative trait loci (molQTL) dataset including; gene expression, open chromatin and enhancer activity in human umbilical vein endothelial cells (HUVEC) from a panel of 100 donors. We integrated this with previously published QTL data from our laboratory generated in three immune cell types; monocytes, neutrophils, and naïve T lymphocytes. We performed colocalisation analysis between molQTLs and 17 cardiovascular disease GWAS, including Coronary Artery Disease and Atrial Fibrillation. In addition, to directly test genetic effects on endothelial barrier integrity we used Electric Cell-substrate Impedance Sensing (ECIS) as a quantitative trait to perform genetic association analysis. We resolved hundreds of risk loci in both endothelial and immune cells, both highly pleiotropic loci that were shared across cell types and traits, and loci that were restricted to cell type relevant pathways. As an example using our molQTL and ECIS data we identified a novel isoform QTL at the IGF2BP3 gene which has recently been shown to protect against endothelial dysfunction.

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P22.031.D Sex/Gender differences in circRNAs expression and function in Multiple Sclerosis

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Background: Multiple Sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) with a female to male ratio up to 3:1. MS exhibits sex differences in immune responses and neurodegeneration, influencing disease susceptibility and progression. Accumulating evidence highlights the key roles of circular RNAs (circRNAs) in regulating CNS and immune cell functions. This study aims to analyze the role of circRNAs in MS pathogenesis, with a particular focus on sex differences and their potential as biomarkers.

Material and Methods: A microarray assay was used to profile circRNA expression in PBMCs from female MS patients (FPwMS) compared to male MS patients (MPwMS). RT-qPCR was performed to validate the expression of selected circRNAs. Additionally, circRNA expression was analyzed in MS patients with varying disease severity and under different drug treatments. Bioinformatics analysis was employed to map interactions between circRNAs and other ncRNAs to identify the competitive endogenous RNA (ceRNA) network.

Results: Among the 14,000 circRNAs detected, 33 were found to be differentially expressed between FPwMS and MPwMS. We deeply validated 1 circRNAs upregulated and 4 downregulated in PBMCs and in serum of FPwMS and MPwMS. Furthermore, the expression levels of these circRNAs were assessed in relation to disease severity and drug treatments. CeRNA analysis identified key mRNAs, miRNAs, and lncRNAs implicated in driving MS pathogenesis.

Conclusion: Our results highlight the implication of circRNAs in MS pathogenesis with a specific focus on sex/gender differences and their potential use as biomarkers. These findings provide new insights into the molecular mechanisms underlying MS.

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P22.032.A Loss of imprinting at the *RB1* locus in families with *RB1* promoter variants supports the role of transcription in establishing oocyte-specific DNA methylation at the *RB1* DMR

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Background: Retinoblastoma (RB) is an autosomal dominant tumour predisposition caused by mutational inactivation of the tumour suppressor gene *RB1*. Parent-of-origin effects on RB penetrance were noted in families with a specific *RB1* variant before *RB1* was found to be imprinted and were later observed for other variants, too. *RB1* imprinting is due to a differentially methylated region (DMR) in intron 2, methylated on the maternal and unmethylated on the paternal allele, where it acts as a promoter for an alternative *RB1*-transcript. Its expression interferes with expression of the regular upstream *RB1*-transcript, leading to skewed expression in favour of the maternal allele. In the growing oocyte, methylation at maternally methylated DMRs occurs via transcription traversing these DMRs. Genetic analysis of families with variants silencing expression of the regular *RB1*-transcript may provide evidence for transcription's role in establishing *RB1*-DMR methylation.

Material and Methods/Results: MS-MLPA analysis of blood DNA of 7 families with pathogenic *RB1* promoter variants revealed that all 4 individuals with a maternally transmitted variant showed hypomethylation at the DMR. All 6 individuals with a paternally transmitted variant showed normal methylation of 50%. Analysis of the RB phenotype distribution in our and published families with variants silencing expression of the regular *RB1*-transcript, showed no dependence on parental origin.

Conclusion: Our observations indicate that establishing methylation at the *RB1*-DMR in the oocyte depends on active transcription through this region. The absence of a parent-of-origin effect in families with silenced *RB1* transcription suggests that this phenomenon is mediated by imprinting at the *RB1*-DMR.

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P22.033.B Variant-to-function dissection of rare non-coding GWAS loci with high impact on blood traits

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Two decades of Genome Wide Association Studies (GWAS) have yielded hundreds of thousands of robust genetic associations to human complex traits and diseases. Nevertheless, the dissection of the functional consequences of variants lags behind, especially for non-coding variants. Here we have characterised a set of rare, non-coding variants (RNVs) with large effects on haematological traits by integrating (i) a massively parallel reporter assay and (ii) in vivo gene expression and transcript relative abundance analysis of whole blood and immune cells. After extensive manual curation we identify 22 RNVs regulating genes robustly associated with the GWAS traits and perform an in-depth characterization of one of them, demonstrating its impact on megakaryopoiesis through modulation of the CUX1 transcriptional cascade. With this work we advance the understanding of the translational value of GWAS findings for variants implicated in blood and immunity.

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P22.034.C Establishing Zebrafish and iPSCs-models of *ANO4* missense variants linked to neurodevelopmental and epileptic disorders

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Background: We previously described missense variants in *ANO4* to result in distinct phenotypes of fever-sensitive developmental

disorders associated with diverse types of epilepsy with or without cognitive impairment. These variants impair ANO4 ion channel and scramblase activity, exhibiting evidence for a dominant-negative effect. Additionally, we observed a heterozygous *ANO4* deletion in a healthy individual suggesting that the disease mechanism is likely linked to dysfunctional protein rather than haploinsufficiency.

Material and methods: To better understand genotype-phenotype correlation, we perform in vivo experiments using zebrafish model. Concurrently, we generate patients-derived iPSCs and their isogenic controls, to then differentiate both into excitatory forebrain neurons.

Results: Sequence alignment revealed that both human *ANO4* and *ANO3* align to the Zebrafish orthologue *ano3*. To test if *ano3* combines functions of both human genes, we generate knock-down fish followed by rescue experiments. *Ano3* knock-down zebrafish displayed developmental and locomotion impairment with 27% of fish exhibiting multi-organs malformations. Preliminary rescue experiments with human *ANO3* mRNA Wt partially restored the phenotype, suggesting that human *ANO4* might also be necessary for full phenotypic rescue. Overexpression of human *ANO3* mRNA Wt led to high lethality (up to 57%) and increased rate of malformations. Overexpression of human *ANO4* mRNA Wt shows a milder impact with a lower lethality rate and less malformed fish.

Conclusions: Our preliminary results suggest that the zebrafish orthologue *ano3* may function as both human *ANO3* and *ANO4*. Knock-down *ano3* affects zebrafish development and neuronal activity. Overexpression of human *ANO3* and *ANO4* independently leads to lethality and malformations.

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P22.035.D The transcription factor FOXM1 reverts DNA damage-driven epigenetic erosion in aged cells

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Background: DNA damage is a key driver of aging, leading to epigenetic erosion, cellular senescence, and chronic inflammation. FOXM1, a transcription factor repressed with age, is recognized as a master regulator of DNA repair. However, its role in mitigating DNA damage and preserving epigenetic integrity in aging remains poorly understood.

Material and Methods: Fibroblasts from young donors were subjected to *FOXM1* knockdown using RNA interference, while FOXM1 overexpression was induced in fibroblasts from aged donors via a lentiviral transduction system. DNA damage and protein levels of epigenetic regulators were measured by immunofluorescence. Gene expression changes were measured using RNA-seq, and changes in binding profiles of histone modifications were examined using CUT&RUN.

Results: *FOXM1* knockdown in fibroblasts from young donors downregulates essential DNA repair pathways, resulting in increased DNA damage. This promotes the proteolytic degradation of the G9a methyltransferase and a subsequent loss of the H3K9me2 epigenetic mark at the nuclear periphery. Consistent with FOXM1 repression during aging, G9a and H3K9me2 levels are reduced in aged fibroblasts. Loss of H3K9me2 at specific loci is associated with increased expression of senescence and inflammatory processes. Remarkably, overexpression of *FOXM1* in aged fibroblasts restores DNA repair gene expression and reduces DNA damage. This reinstates both G9a and H3K9me2 levels to repress genes involved in inflammation and senescence.

Conclusion: These findings establish FOXM1 as an age reversal factor capable of restoring epigenetic integrity by enhancing DNA repair, offering a promising therapeutic avenue to address the fundamental causes of aging.

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P22.036.A Single-cell analysis of epigenomic changes induced by culture adaptation in human embryonic stem cells

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Background: Long-term culture of H9 human embryonic stem cells (hESCs) leads to genomic and epigenetic alterations, including changes in chromatin accessibility, copy number variations (CNVs), and gene expression. Epigenetic changes play a critical role in regulating gene expression and cellular behavior during culture adaptation. These changes may give rise to culture-adapted phenotypes, such as increased survival and proliferation.

Material and Methods: Single-nuclei ATAC-seq (snATAC-seq) was conducted across four passage stages (P1-P4) to profile chromatin accessibility. Cis-coaccessibility networks and functional enrichment analyses identified key differentially accessible regions (DARs). Transcription factor (TF) activities and chromatin accessibility-based CNV estimations were also assessed.

Results: Long-term culture of hESCs resulted in distinct chromatin accessibility profiles, with elevated accessibility in high-passage populations (P3/P4) linked to centrosome organization genes. In Chr20q11.21, specific regions exhibited RNA expression patterns that correlated more strongly with changes in open chromatin rather than CNV. TEAD (transcription enhanced associate domain) family TFs were identified as key regulators of BCL2L1 and TPX2, with increased activity in higher passages.

Conclusion: Prolonged culture induces significant genomic and epigenetic adaptations, characterized by distinct transcriptional and chromatin changes that drive culture adaptation and survival phenotypes. Particularly, these changes in TEAD-regulated genes contribute to spindle assembly and anti-apoptotic phenotypes, with cell line-specific variability during culture adaptation. These findings provide valuable insights into mechanisms underlying hESC culture adaptation and highlight potential implications for maintaining genomic integrity.

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P22.037.B Lead exposure alters global DNA methylation in pediatric Cystic Fibrosis patients from Georgia

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Background: Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in Caucasians that mainly affects the respiratory and gastrointestinal systems [1]. Genotype-phenotype correlations in CF twins showed that modifier genes and environmental factors also contribute to phenotypic variability in CF patients [2, 3]. Lead (Pb) is a pervasive environmental neurotoxin that does not degrade or lose potency over time, contaminates population centers worldwide, and has no safe level of exposure for children [4]. In this study we examined plasma levels of lead and analyzed global DNA methylation markers in Georgian CF patients.

Material and Methods: The study was approved by the Ethics Committee of the Tbilisi State Medical University (Tbilisi, Georgia). A total of 34 patients (age 5.5 ± 3.8) were recruited in this study. DNA was treated using the EZ DNA Methylation-Gold Kit (Zymo Research, CA, USA) according to the manufacturer's protocol. LINE-1 and Alu methylation levels were evaluated using COBRA-IRS method. The plasma lead level was quantified using atomic absorption spectrometry (Agilent 240FS AAS, USA).

Results: The mean plasma lead levels was 2.6 µg/dL. Pb exposure was correlated with methylation of the LINE-1 and Alu elements. Significantly decreased methylation was found in both elements in children with high plasma Pb compared to low plasma Pb ($p < 0.05$).

Conclusion: This study was a first try to correlate methylation status of the LINE-1 and Alu retrotransposons with plasma Pb levels in CF. The result suggests that Pb exposure increases aberrations in DNA methylation in Cystic Fibrosis.

C.O.I. Disclosure:

S. Surmava: None. **T. Tkemaladze:** None. **N. Dabrundashvili:** None. **T. Atamashvili:** None. **M. Gagua:** None. **E. Abzianidze:** None. **E. Kvaratskhelia:** None.

P22.038.C Identification of circular RNA associated with human traits

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Background: Circular RNAs (circRNAs) are single-stranded non-coding RNA depicted by a covalently closed circular structure; circRNA are generated from pre-mRNAs through back-splicing process. Recently, with the progression of high-throughput sequencing technologies, several circRNAs have been identified, and functional analyses illustrated their effect on human diseases including autoimmunity. However, studies on the function of circRNAs in immune response and autoimmunity are still lacking.

Material and Methods: Here, we present a collection of 67 total leucocyte RNA-seq datasets that were used to explore the cellular and humoral landscape of circRNAs in human samples. Using a custom pipeline for cirRNA calls exon-exon junctions we identify circRNAs expressed in the RNA-seq libraries. Next, using correlation and regression analysis, we link circRNAs with immune-related phenotypes available from the SardiNIA cohort.

Results: We identified a total of 54 specific circRNA expressed in leukocytes. Among them, 7 signals were significantly associated with immune cells levels, 9 with inflammatory molecules, and 2 showing a different behavior in males and females. Of note, we revealed a correlation between hsa_circ_0001947 levels, a circular RNA previously associated with Multiple Sclerosis, and granulocyte levels. Further analysis showed that hsa_circ_0001947 is upregulated in our Multiple Sclerosis data set and was further analyzed.

Conclusion and future prospective: This study expands our knowledge of circRNA expressions and correlation of circRNA levels with immune traits and provides a useful resource for exploring circRNAs in autoimmunity. Ongoing analyses are focused on understanding hsa_circ_0001947 role in MS and to deeply analyzed the levels of genetic regulation for circRNAs.

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M.L. Idda: None. **M. Steri:** None. **M. Masala:** None. **V. Lodde:** None. **M. Pala:** None. **F. Cucca:** None.

P22.039.D Impact of ketogenic diet treatment in GLUT1 deficiency syndrome: identification of molecular mechanisms related to phenotype changes in both pediatric and adult patients

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GLUT1 deficiency syndrome (GLUT1-DS) is a rare drug-resistant epilepsy caused by mutations in *SLC2A1*, encoding GLUT1, which supplies glucose to the brain. A genetic defect leads to cerebral energy deficiency, resulting in a complex spectrum of neurological symptoms. Ketogenic diet (KD) provides alternative sources for metabolism, whereas its mechanisms remain still unclear. This study aimed to investigate clinical variability and wide spectrum of *SLC2A1* variations, phenotype age-related changes, response to KD through a multi-omics approach. Analyses of 27 patients allowed the identification of different clinical phenotypes, 25 variants of which 10 novel, and correlations between variations, clinical and biochemical patterns. We explored the transcriptional profiles focusing on (a) adults versus children, both untreated; (b) children in pre- versus post-KD; (c) diet responders versus non-responders. Enrichment analysis revealed a reduction in inflammatory pathways in patients who experienced clinical improvements, whether in response to KD or during adulthood. These processes involve type I IFN signatures, which have an impact on mitochondrial signaling, suggesting a role in GLUT1-DS. Supporting these suggestions, the identified lncRNAs are related to inflammatory response, or to ketone biosynthesis process, like PAX8-AS1, for which emerges its potential role in KD response. Another pathway contributing to mitigate clinical signs is the alternative splicing of ion channel genes: P2X7 isoforms which are, in turn, linked to inflammasome. Concurrently, methylome profiles, performed by ONT, revealed altered methylation patterns in similar genes. These novel findings underline the complex interplay among transcriptional profiling, isoform switching and methylation patterns in the unresolved aspects of GLUT1-DS.

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P22.040.A Epigenetically regulated genes and pathways associated with nonsyndromic cleft lip and palate identified through genome-wide DNA methylation analysis

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Background: Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is the most common and severe craniofacial disorder, with both genetic and environmental causes. NSCL/P is linked to increased morbidity and mortality in childhood and adulthood. While genetic mechanisms of familial NSCL/P have been studied, epigenetic factors are increasingly implicated in orofacial development.

Materials and methods: To explore this, genome-wide DNA methylation analysis was performed on blood samples from 48 individuals, including 20 NSCL/P cases and 21 controls, using the Illumina Infinium MethylationEPIC array.

Results: Comparison of methylation profiles revealed 528 differentially methylated CpG loci, associated with 606 genes showing at least 8.0-fold methylation change, FDR $p < 5 \times 10\text{-}8$, and AUC ≥0.75. Notable genes identified include COL4A1, TP63, MSX1, PAX6, WNT6, and others previously linked to NSCL/P. Pathway analyses indicated that altered signaling in pathways such as growth hormone, Cdc42, GABA receptor, P38 MAPK, Wnt-B catenin, and BMP are associated with NSCL/P risk.

Conclusion: These findings support that NSCL/P is linked to differential DNA methylation in whole blood, which could serve as biomarkers for NSCL/P risk.

C.O.I. Disclosure:

A. Vedangi: None. **U. Radhakrishna:** None.

P22.041.B Reduction of the number of CpG dinucleotides required for episignature analysis in *CREBBP*-related Rubinstein-Taybi syndrome

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Background: Genome-wide DNA methylation signature analysis, also known as episignature analysis, has proved to be an effective second-tier diagnostic tool for a number of chromatinopathies. However, there are several diseases, including CREBBP-related Rubinstein-Taybi syndrome (RTS1), for which genome-wide DNA methylation changes cannot be considered robust enough. We aim to represent an approach to the reduction of the RTS1 episignature which leads to an increase in classification accuracy.

Material and Methods: A total number of 99 samples were analysed, including the following DNA samples: 60 from patients with established pathogenic or likely pathogenic variants in the CREBBP gene, 5 from patients with detected variants of uncertain significance in the CREBBP gene, 4 from patients with syndromes phenotypically overlapping with Rubinstein-Taybi syndrome and 30 from healthy controls. Targeted methods based on high-throughput bisulfite sequencing and Sanger bisulfite sequencing were applied to measure the methylation level of the selected loci.

Results: Using machine learning techniques, we established that chr6:10556199_chr6:10556204 locus is the most significant predictor among those we had initially selected. The classification models based on targeted DNA methylation analysis methods set apart RTS1 and non-RTS1 samples with values of accuracy ≥0.9. Moreover, reduced episignature analysis shows utility in classifying samples with variants of uncertain significance in the CREBBP gene.

Conclusion: The proposed concept is quite promising in terms of increasing RTS1 detection and can be implemented on other chromatinopathies with known genome-wide episignatures.

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P22.042.C In silico and molecular investigation of genomic interactions reveals shared pathways contributing to phenotypic overlap in neurodevelopmental disorders

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Background: Neurodevelopmental disorders can result from variants in genes that participate in the epigenetic machinery. Patients with clinical suspicion of such conditions often present overlapping phenotypes such as intellectual disability, facial dysmorphisms, and ophthalmological abnormalities, posing challenges for research diagnostics. This study aims to investigate the phenotypic overlap among different conditions through in silico analysis of genes identified by Whole Exome Sequencing (WES) and their interactions, providing deeper insights into the genetic mechanisms underlying these disorders.

Material and Methods: Forty patients with a phenotype suggestive of neurodevelopmental disorders with normal cytogenomic tests were studied by WES. The detected gene variants were classified following the recommendations of the American College of Medical Genetics and Genomics (ACMG), followed by genotype-phenotype correlation. Gene interaction analysis was performed with in silico tools (STRING, GeneMANIA, BioGRID, Enrichr, and Cytoscape).

Results: WES results of five patients showed pathogenic and likely pathogenic variants in *CHD3*, *ARID1B*, *CREBBP*, *DNMT3A*, and *KAT6B*. These genes are involved in chromatin modulation and significantly impact the epigenetic machinery. A genotype-phenotype correlation was established for these patients, highlighting overlapping phenotypic features. In silico analysis revealed distinct interactions among these genes, which may explain the phenotypic similarities observed and the difficulty of an accurate diagnosis.

Conclusion: WES is an efficient technique to improve the diagnosis of patients with altered phenotypes and normal cytogenomic results who are under the suspicion of neurodevelopmental disorders. Moreover, WES allows the identification of genes that interact with each other, potentially guiding the diagnostic research of such patients.

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P22.043.D Alternative splicing during differentiation contributes to differences in the response to type I interferon observed in monocytes and macrophages

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Background: Monocytes are able to enter tissues and rapidly differentiate into macrophages in response to the microenvironment. During this process, cells undergo transcriptional changes that reflect the differences in their functions, which include differences in their response to cytokines such as type I interferons (IFNs). We therefore sought to examine how changes occurring during the differentiation process impact cytokine responses in the resulting cells.

Material and Methods: Monocytic THP-1 cells were treated with phorbol 12-myristate 13-acetate to induce differentiation into macrophages. Differentiation was confirmed using flow cytometry and microscopy. Monocytes and macrophages were then treated

with type I IFN (IFN-α) and RNA sequencing was performed. Changes in RNA abundance and isoform expression were evaluated before and after type I IFN treatment in both monocytes and macrophages. Selected alternative splicing events were validated using RT-qPCR.

Results: Monocytes and macrophages share overlap in their response to type I IFN with respect to changes in gene expression, but considerable differences are also observed between these cell types. These occur at both the level of gene expression and in the mRNA isoform profiles of each cell type. Even where genes are differentially expressed in response to type I IFN in both cell types, differences in alternative splicing diversify the response between cell types. These alternative splicing events can often be attributed to changes arising during the process of monocyte-to-macrophage differentiation.

Conclusion: This study highlights the role of alternative splicing events occurring during monocyte-to-macrophage differentiation in determining cell type-dependent responses to type I IFN.

C.O.I. Disclosure:

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P22.044.A Effects of PFAS on DNA methylation at human cancer susceptibility loci

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Background: Per- and polyfluoroalkyl substances (PFAS), including perfluorooctane sulfonate (PFOS), are persistent pollutants linked to increased risks of cancers and other diseases. These chemicals accumulate in biological systems and potentially disrupt epigenetic regulation. Our study focuses on the effects of PFOS on DNA methylation at risk loci for human cancers, with a hypothesis that such effects may be mediated by inflammatory signaling.

Material and Methods: Immortalized human fallopian tube epithelial (FNE) and B lymphoblastoid (GM12878) cells, modeling the precursors of ovarian cancer and B cell malignancies, were exposed to PFOS (0, 10, 25, 50 μM) or TNF-alpha (0, 50, 100, 200 ng/mL) for 48 hours. Targeted methyl-seq was performed to assess changes in net and allele-specific methylation patterns in a panel of GWAS-defined cancer susceptibility loci.

Results: In FNE cells, PFOS exposure induced concentration-dependent changes in net and allele-specific methylation spanning a regulatory SNP in the HOXD gene cluster associated with ovarian cancer. In GM12878 cells, PFOS exposure affected methylation in the S100A gene cluster and HNF1B, associated with multiple myeloma, as well as FGFR2, linked to breast cancer. Similar epigenetic changes were observed in TNF-alpha-treated cells, suggesting that inflammatory signaling may act as a mediator in PFOS-induced epigenetic alterations.

Conclusion: Our findings demonstrate that PFOS exposure alters DNA methylation at disease risk loci and suggest that such effects might be mediated through inflammatory signaling. Ongoing work includes functional tests of the inflammatory pathway hypothesis and assessing effects of PFAS at lower concentrations over longer exposures.

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P22.045.B Phenome-Wide Association Study and Functional Annotation of Hemoglobin A1c-Associated Variants in African Populations

C. Soremekun[1], *O. O. Ojewunmi*[2], *A. Nwagbata*[3], *H. Bazireh*[4], *S. Sharma*[5], *D. Jjingo*[6], *D. Kateete*[6], *O. Nash*[7], *H. Grallert*[5], *C. Batini*[8], *A. Peters*[5], *S. Fatumo*[2];

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Background: Glycated hemoglobin (HbA1c) measures the average blood sugar level over the past three months. As a vital biomarker of blood glucose levels, it is used to diagnose Type-2 diabetes mellitus (T2D) and monitor glycemic control. Therefore, we performed a Phenome-Wide Association Study (PheWAS) and functional annotation of HbA1c-associated variants in two African populations.

Method: In this study, we utilized summary statistics of the HbA1c GWAS meta-analysis of 7,526 individuals from South Africa and Uganda to conduct a PheWAS using GWASATLAS. We also performed a functional analysis using FUMA tool. SNPs were prioritized using the SNP2GENE function, while the genes' expression patterns and shared molecular functions were explored in the GENE2FUNC.

Result: The functional annotation results identified three genome-wide significant loci with the lead SNPs rs6724428, rs148228241, and rs8045544 mapped to *GULP1*, *HBA1*, and *ITFG3* genes. The minor allele frequency for rs148228241 is 0.074 and is exclusive to African populations. Both rs8045544 and rs148228241 are significantly associated with the mean corpuscular hemoglobin concentration (MCHC). The rs6724428 variant is significantly associated with skeletal functions, reflecting the link between glucose metabolism and bone mineral density.

Discussion: Our findings highlight the intricate relationships between glucose metabolism, erythropoiesis, and skeletal health. The significant associations of HbA1c-variants with both skeletal function and MCHC underscore the potential of these variants to impact broader physiological processes. A larger study involving African individuals will be necessary to validate the link between the *HBA1* gene and HbA1C.

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P22.046.C Classification of Three VUS Variants Using a Functional Laboratory Testing

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Background: Lynch syndrome is a heritable condition caused by a heterozygous germline mutation of the DNA mismatch repair genes, most commonly the *MLH1* gene [1, 2]. However, one third of the identified alterations are missense variants, for which the clinical significance is unclear in many cases [3, 4]. We have identified three *MLH1* missense alterations from Tunisian families. The classification of pathogenicity was not available for these variants, consequently diagnosis, predictive testing and targeted surveillance in affected families was impossible.

Material and Methods: This study examines three variants of uncertain significance identified in six patients with suspected Lynch syndrome. For the first time in Tunisia, we conducted functional laboratory assays to evaluate both protein stability and catalytic activity, utilizing a system incorporating clinically validated reference variants.

Results: Both p.(Leu73Pro) and p.(Pro640Thr) were found to be non-functional due to severe defects in protein stability and catalytic activity. In contrast, p.(Glu736Lys) was comparable to the wild-type protein and therefore considered a neutral substitution. Analysis of residue conservation and of the structural roles of the substituted residues corroborated these findings. In conjunction with the available clinical data, two variants fulfil classification criteria for class 4 "likely pathogenic".

Conclusion: In conjunction with clinical data of our patients, the functional results allow for classifying two of the found variants into class 4 (likely pathogenic), while no functional defects could be found in another variant. These findings will improve genetic counselling and clinical management of affected Tunisian families and other carriers of these genetic variants worldwide.

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P22.047.D Dysregulated non-coding RNAs drive preeclampsia, revealing molecular mechanisms and diagnostic potential

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Background: Preeclampsia (PreE) is a pregnancy-related disorder characterized by high blood pressure and organ dysfunction, affecting both mothers and infants. It can impair the liver, kidneys, brain, and clotting system. While its global prevalence is 2%–10%, it affects 13%–15% of pregnancies in India. Emerging research links non-coding RNAs, including microRNAs (miRNAs), to pregnancy complications like PreE. This study investigates their role and potential as biomarkers for early detection and monitoring.

Material and Methods: Genome-wide DNA methylation analysis was performed on 20 women with severe preeclampsia and 20 normotensive controls using the Illumina Epic Array platform. Statistical and bioinformatic approaches were integrated to identify differentially methylated microRNAs associated with preeclampsia.

Results: The study identified 540 significantly differentially methylated CpG sites in 540 genes associated with preeclampsia (preE). Among these, 45 miRNA CpG sites exhibited significant methylation changes in preE and had been previously reported as being associated with the condition. These include miRNAs such as miR-675, miR-210, miR-378a, miR-411, miR-744, miR-519E, miR-525, miR-100, miR-328, miR-127, miR-379, miR-377, miR-195, miR-376c, and miR-378A. These dysregulated miRNAs are expected to target multiple coding genes, potentially contributing to preE development by regulating key cellular processes such as trophoblast proliferation, migration, invasion, apoptosis, differentiation, metabolism, and angiogenesis.

Conclusion: This study reveals significant miRNA methylation changes in preeclampsia, highlighting their potential as predictive biomarkers. These findings open avenues for personalized treatments, though further research is needed to confirm their diagnostic and therapeutic value. Exploring their target genes could deepen our understanding of preeclampsia's molecular mechanisms.

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P22.048.A A new mechanistic key to functionally interrogate the regulatory genome

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Most signals in genome-wide association studies (GWAS) are found in the noncoding regions of the genome, yet functional mechanisms to decode them are lacking. We recently described a new mechanism that controls gene expression of the *RHODOPSIN* (*RHO*) gene by combining perturbations of *cis*-regulatory elements (CREs) in "*trans*" and "*cis*." We found that by binding a short CRE within the RHO promoter with synthetic DNA-binding proteins (in *trans*), delivered into the retina by adeno-associated viral vectors (AAVs), it is possible to control the expression levels of the RHO gene (Botta et al., 2016). Furthermore, with an in vivo reporter assay carried out with AAV vectors, we have shown that the DNA sequence that the synthetic DNA-binding proteins bind in *trans*, when modified in *cis*, results in a wide variation in reporter gene expression outputs (Maritato et al., 2024). Specifically, we found that the composition and length (22 bp) of the DNA sequence interposed between two retinal transcription factors (TFs), CRX and NRL, largely controls RHO expression levels. These *trans* and *cis* perturbations argue that, in this case, the DNA sequence itself interposed between CRX and NRL contributes to their activities through a code based on specific DNA sequence of defined length, ultimately determining the levels of RHO expression. These studies may provide a new mechanistic key to functionally interpret the regulatory genome. This opens avenues to decode noncoding genetic variants across the genome and to exploit cis- and trans-regulation to encode novel therapeutic strategies.

C.O.I. Disclosure:

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P22.049.B Telomere shortening, oxidative stress products, and related-miRNAS as biomarkers in the response to mild SARS-CoV-2 infection

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Background: In infectious diseases, oxidative stress, and inflammation can modulate immune response and epigenetic machinery. Host-encoded miRNA profiles in mild COVID-19 are poorly investigated, although they can reveal information about oxidative stress and senescence on miRNA expression during disease. We evaluated ageing biomarkers, such as telomere shortening, oxidative stress indicators (TBARS and 8-OHdG), and related-miRNAs expression in response to viral infection.

Material and Methods: The study included 75 individuals with mild SARS-CoV-2 infection and 30 healthy controls. Telomere length (TL) was measured in DNA from whole blood and saliva samples. Oxidative stress biomarkers were quantified in plasma through spectrophotometry. Ageing and oxidative stress-related miRNAs (miR-155-5p, miR-34a-5p, miR-138-5p, miR-210a-3p, and miR-182-5p) expression in serum samples were assessed by qRT-PCR, followed by an in-silico (IS) analysis.

Results: TL correlated with age ($R = -0.384$, $p = 0.005$), and was shorter in infected individuals (rTL 1.46 ± 0.51 vs. 0.99 ± 0.37; $p < 0.001$). rTL was similar between the tissues analyzed ($p = 0.917$). 8-OHdG was increased in cases ($p = 0.040$), with no differences in TBARS concentrations ($p = 0.934$) compared to controls. miR-138-5p was upregulated in COVID-19 cases ($p = 0.026$) and positively correlated with 8-OHdG ($R = 0.403$, $p = 0.05$) indicating its involvement in viral processes (GO:0016032, p = 1.3e-08; in-silico). miR-210-3p was overexpressed in controls ($p = 0.008$) and miR-182-5p expression correlated with TBARS levels ($R = 0.582$, $p = 0.018$).

Conclusion: The relationship between TL and DNA damage in mild COVID-19 cases may indicate early systemic cellular damage processes. miR-182-5p might serve as an early biomarker of oxidative stress in COVID-19. Overexpression of miR-138-5p could represent a compensatory mechanism against damage caused by the infection.

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P22.050.C Multiple Sclerosis patients show a depletion in Gut Microbiota keystone *genera* compared with their cohabiting Healthy Controls: a pilot study

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Background: In the complex pathophysiology of Multiple Sclerosis (MS) gut microbiota (GM) and dietary habits have been identified as significant contributors of disease development. GM dysbiosis is accompanied by alterations of immune system and immune response, due to the intricate link between GM composition, metabolite production and immune system stimulation.To minimize confounding factors, healthy controls (HC) who cohabit with MS patients have been recruited. The present study aimed at identifying possible alterations in the GM of MS patients through the 16S rRNA sequencing.

Material and Methods: 24 relapsing-remitting MS patients and healthy controls have been recruited for the study. A seven-day food diary has been administered to subjects, and food intake has been analyzed through MetaDieta software. GM composition has been characterized by sequencing the V3-V4 hypervariable regions of the 16S rRNA gene. Bioinformatic analysis have been conducted using DADA2 and Microbiome Analyst.

Results: Overall, no significative differences between MS patients compared with HC in total energy intake as major nutrients have been found.Alpha diversity metrics showed a decreased biodiversity in MS compared to HC, while no significant clustering has been found in beta diversity. MS patients show an enrichment in *Oscillibacter, Romboutsia* spp. and UBA1819, and a depletion in *Faecalibacterium, Roseburia* and *Coprococcus*, the major butyrate producing genera.

Conclusion: The absence of dietary differences between MS and HC suggests that recruiting household members as controls can minimize the impact of dietary variations between the groups.Our findings align with the existing literature, reinforcing the understanding of GM alterations in MS.

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P22.052.A Reduced episignature of Sotos syndrome

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Background: Episignature is usually detected by genome-wide methods using the Infinium MethylationEPIC microarray. In our work, we reduced the episignature of Sotos syndrome to the two most representative CpG sites and developed a classification model. This reduction allows detection of the episignature with targeted bisulfite sequencing.

Material and Methods: The most significant predictors (CpGs) in terms of classification ability were searched using Random Forest method with the Boruta algorithm. We collected DNA samples from 26 patients with pathogenic/likely pathogenic (P/LP) variants in the *NSD1* gene, 2 patients with variants of uncertain significance (VUS) in *NSD1*, 3 patients with likely pathogenic variants in *NFIX* and 11 control samples. Target CpGs methylation was assessed using bisulfite Sanger sequencing and local pipeline. The classification model was created by the support vector machine with cross-validation. The quality of the model was assessed using ROC analysis.

Results: Multiple classification models were built using single predictors and their possible combinations. The classification model based on the combination of predictors cg22497095 and cg20851828 showed the best sensitivity (99%), specificity (93%) and accuracy (94%) and was selected for further use. All samples with P/LP and VUS variants in *NSD1* were classified as Sotos syndrome samples. Control and *NFIX* samples were classified as normal samples.

Conclusion: Sotos syndrome episignature may be effectively reduced without significant loss of diagnostic significance.

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P22.053.B Genes aberrantly methylated in NSCLC and CIS are enriched for olfactory transduction genes

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Background: Lung cancer is the leading cause of cancer-related deaths[1]. Epigenetic changes, like DNA methylation, are key to understanding tumor biology and biomarker discovery. This study examines methylation patterns to uncover differences by tissue type and medical history.

Material and Methods: A retrospective analysis was conducted using the Gene Expression Omnibus database[2], incorporating 982 patients from five studies across three continents. The dataset included 244 normal and 54 carcinoma in situ (CIS) samples. Eligible studies were identified through a review of 937 publications (up to January 2024) using HumanMethylation 450K Array data. Differential methylation analysis (DMA) was conducted via the Wilcoxon rank-sum test (MWW).

Results: DMA was performed between normal and pathological samples, histological subtypes, and subgroups stratified by medical history. Methylation of a core set of 256 loci was consistently altered across multiple analyses (MWW, variance analysis, PCA) and ranked within the top 3000 results. Pathway enrichment analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) revealed significant alterations in the DNA methylation status of genes contributing to olfactory transduction in all samples (GO $p = 4.2 \times 10^{-13}$) and in loci associated with tumor recurrence (GO $p = 1.5 \times 10^{-13}$, KEGG $p = 1.7 \times 10^{-15}$). DMA between Short-Term Relapsers and Long-Term Survivors yielded no significant results.

Conclusion: The study identifies significant epigenetic differences between lung cancer subgroups. Olfactory transduction emerged as a key feature of methylation changes, especially in tumors that later relapsed. These findings reinforce the role of altered olfactory transduction pathways in cancer methylation and suggest a potential role in relapse prediction.

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J. Fend: None. **O. Ammerpohl:** None.

P22.054.C Methylation profile analysis: Evaluation of long read sequencing analyses for routine clinical use

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Background: DNA methylation, an epigenetic marker, can contribute to tumorigenesis by inactivating tumor suppressor genes. In routine diagnosis, gene methylation is in most cases assessed by fragment using methylation-specific enzymes, or sequencing, after modification of unmethylated cytosines by bisulfite reaction. This method can damage DNA, or induce a conversion bias interfering with methylation analysis. Furthermore, these techniques don't allow assessment of an entire CpG island, nor do they allow sufficient sequencing depth for methylation rate assessment, or easy haplotypes phasing.

Material and Methods: These led us to evaluate two emerging technologies, to improve methylation profiling. As an alternative to bisulfite conversion, we tested first the enzymatic conversion of New England Biolabs™: Enzymatic Methyl-seq (EM-seq™), followed by PCR amplification and NGS sequencing of the region of interest. This approach is quantitative and uses a conversion method expected to reduce DNA fragmentation. The second technology tested is Oxford Nanopore long-read sequencing, which enables native DNA to be analyzed after CRISPR/Cas9 enrichment, or after adaptive sequencing. Both have been evaluated on a cohort of 6 DNA samples with methylation profiles of the SDHC promoter, including one germline mosaicism, previously characterized by pyrosequencing after bisulfite treatment.

Results: The two techniques revealed good coverage of the region of interest and showed correlations between the profiles obtained by NGS after NEB conversion versus Nanopore sequencing. Moreover, long read sequencing allows to easily phase methylation haplotypes in imprinting regions.

Conclusion: These results demonstrate the feasibility of the various techniques, and open the discussion on possible routine hospital use.

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P22.055.D Impact of SDHA Mutations on Yeast Growth and Mitochondrial Function. Case Study Linking Genetic Findings to Clinical Phenotypes

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Background: Biallelic mutations in the *SDHA* gene (MIM *600857), encoding the catalytic subunit of succinate dehydrogenase (SDH), are known to cause mitochondrial dysfunctions such as Leigh syndrome (LS), an autosomal recessive neurodegenerative disorder that primarily affects children, and it is characterized by developmental delay, lactic acidosis, ataxia, and seizures. SDHA mutations can present with variable and atypical features, complicating diagnosis. Yeast models (*Saccharomyces cerevisiae*) are used to study *SDHA* mutations and confirm their pathogenicity through complementation assays.

Material and Methods: We report a 15-year-old girl who showed neurodevelopmental regression, focal seizures, and behavioral disturbances. Genetic analysis included a trio whole genome sequencing (WGS) to identify mutations, whereas a muscle biopsy was performed to assess mitochondrial function. Functional studies in yeast helped to evaluate the pathogenicity of identified *SDHA* mutations using growth assays and enzyme activity measurements.

Results: WGS identified two heterozygous variants in *SDHA*: c.511C>T (p.Arg171Cys), classified as likely pathogenic, and c.1393C>T (p.Arg465Trp), of unknown significance. Both variants affect conserved amino acids in yeast Sdh1. Functional assays in *Saccharomyces cerevisiae* showed that the p.Arg171Cys mutation significantly impaired growth on glycerol, whereas p.Arg465Trp exhibited hypomorphic behavior. These findings support the pathogenicity of both variants and validate yeast as a model for mitochondrial dysfunction studies.

Conclusion: This case underscores the clinical variability of *SDHA*-related disorders and the importance of functional assays in confirming genetic findings. A comprehensive diagnostic approach combining genetic, biochemical, and functional evaluations is crucial in rare mitochondrial disorders, where traditional methods may be inconclusive.

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P22.056.A Hidradenitis Suppurativa is associated with Non-Alcoholic Fatty Liver Disease due to dysregulated DNA methylation

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Background: Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder caused by fat accumulation in liver cells, leading to inflammation and damage. NAFLD is significantly more prevalent in hidradenitis suppurativa (HS) patients (72.9%) than in controls (24.7%). It progresses through stages from steatosis to cirrhosis, increasing the risk of liver failure and cancer. Obesity, insulin resistance, high cholesterol, and genetic factors contribute to its development. Low vitamin D levels and metabolic syndrome further elevate the risk.

Materials and Methods: Genome-wide DNA methylation analysis was conducted on 24 HS cases and 24 healthy controls using the Illumina EPIC Array platform. Statistical and bioinformatics approaches were employed to identify differentially methylated CpG sites associated with NAFLD.

Results: The study identified 25 differently methylated CpGs in 25 genes, including ABCC2, CPN1, CWF19L1, ENPP1, FTO, GCLC, IRS1, LEPR, LPIN1, MAPK10, MTTP, NR1I2, PEMT, SLC27A5, SOD2, TCF7L2, HIC1, APC, DPP4, WHSC1, MAML3, FLCN, FADS2, and BCAT1, all of which were hypomethylated except for HIC1 and APC. Dysregulation of these genes contributes to NAFLD in individuals with HS by promoting insulin resistance, altered lipid metabolism, inflammation, oxidative stress, and hepatic fat accumulation. These factors interact with the chronic inflammation observed in HS, potentially exacerbating liver injury and fibrosis, leading to the progression of NAFLD.

Conclusion: This study identifies significant methylation changes in key genes in HS, suggesting their potential as predictive biomarkers. These findings lay the foundation for personalized treatment approaches. Further research is needed to validate their diagnostic and therapeutic potential.

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P22.057.B miRNA transcriptome in treatment resistant depression

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Background: Major depressive disorder (MDD) is among the most prevalent mental health conditions, impacting approximately 6% of the global adult population annually. Currently available therapy for depression involves pharmacotherapy combined with psychotherapy, while dealing with poor response/nonresponse and a frequent discontinuation of treatment. The objective of the study was to give better insights into the efficacy and molecular mechanisms behind the effects of alternative methods of treatment in patients with treatment-resistant depression such as transcranial magnetic stimulation (TMS).

Material and Methods: Peripheral plasma from 11 subjects with treatment resistant depression before and after the treatment was used for library preparation for miRNA transcriptome deep sequecing on Illumina NextSeq 550 with 75 bp single-end read. miND analysis pipeline was used for NGS data processing. Statistical analysis of the preprocessed NGS data was conducted using R v4.0, along with the packages pheatmap vNA, pcaMethods v1.82, and genefilter v1.72. Differential expression analysis was performed using the quasi-likelihood negative binomial generalized log-linear model functions from the edgeR v3.32 package.

Results: In the exploratory profiling we identified over 774 mature miRNAs in each sample. Ten most abundant miRNAs in both pre- and post-treatment samples based on their average RPM (reads per 1 million microRNA reads) values were hsa-miR-451a, hsa-miR-486-5p, hsa-miR-16-5p, hsa-miR-92a-3p, hsa-miR-19b-3p, hsa-miR-103a-3p, hsa-miR-25-3p, hsa-miR-15a-5p, hsa-miR-21-5p and hsa-miR-93-5p, each with an RPM exceeding 20,000, and they constitute the core miRNome found in the peripheral blood of subjects with depression.

Conclusion: Identified miRNAs represent starting point for further exploration of changes in transcriptome of MDD.

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P23 New Technologies

P23.001.C CaseSharing: A web service for enhancing rare disease case sharing with Phenopackets

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Background: The cost of whole genome sequencing has been decreasing, making it more common in diagnosing rare diseases. Sharing case information and those with rare variants is crucial for discovering novel relationships between diseases and their associated variants or genes. Additionally, sharing case information can aid in the diagnosis of undiagnosed cases by enabling clinicians to find similar cases and compare clinical features. A federated approach connects national genome initiatives, but much case information remains isolated within clinical settings. To bridge this gap, we propose a user-based system for direct case information sharing in a standardized format. To enhance interoperability, we have utilized Phenopackets, a standard for phenotype and genetic data representation.

Methods: We developed CaseSharing (https://pubcasefinder.dbcls.jp/casesharing), a web service for managing rare disease case information. Case data are stored locally in the JSON format for privacy and security. Additionally, CaseSharingDB collects metadata—such as gene and disease names, and phenotypes—without personal data. With Phenopackets support, users can structure case information in the widely accepted format, enabling seamless sharing and integration with other clinical and research databases.

Results: A key feature of CaseSharing is its Phenopackets support, allowing interoperability between clinical and research platforms. Since case data remains local, there are no concerns about crossing national borders. Only metadata, which excludes personal details, the used for matching between users.

Conclusion: By supporting Phenopackets, CaseSharing improves rare disease research through structured and standardized data sharing. This user-based approach enhances privacy while enabling broader collaboration and facilitating novel disease-gene and disease-variant discoveries.

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P23.002.D Swift and robust detection of tumor fusions: the potential of Nanopore adaptive sampling

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Background: The classification for central nervous system tumors and sarcomas emphasizes the importance of combining anatomopathological, immunohistochemical, and molecular biological markers to achieve the most accurate diagnosis. This approach increases the number of techniques required to characterize tumors and extends the time needed to reach a diagnosis. Innovative techniques capable of providing comprehensive tumor characterization within one single tool are crucial.

Material and Methods: We present approximately fifteen examples of the application of Nanopore sequencing with adaptive sampling targeted enrichment in various cases and tumor types, with a focus on brain tumors and sarcomas, encountered at the Curie Institute.

Results: These examples underscore the significance of Nanopore technology as a rapid and robust tool for tumor characterization, enabling the generation of detailed copy number profiles, the classification of tumor entities through global methylation analysis, and the detection of clinically relevant fusions. This easy-to-implement, flexible technique can be applied to a variety of tumor types without the need for multiple complex library preparations. The workflow is optimized to minimize technical time, and the launch of the bioinformatics pipeline is automated, enabling robust tumor classification in less than 10 days.

Conclusion: Bioinformatics optimizations pave the way for promising applications of Nanopore sequencing. The Sturgeon tool enables methylation classification from extremely small quantities of tumor DNA and could be used to study FFPE samples or circulating tumor DNA extracted from CSF. This application is particularly valuable when the tumor is inaccessible to surgery, facilitating the most accurate diagnosis within a reduced timeframe.

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P23.003.A Performance and Utility of Illumina Protein Prep Proteomics Assay in Rare Disease Clinical Research

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Background: Better methods are needed to increase the proportion of patients with rare diseases who are referred to genomic medical services and receive a successful diagnosis.

Material and Methods: We evaluated novel proteomic assays combining Standard Biotool's (SBI, formerly SomaLogic) SomaScan technology and Illumina Protein Prep to measure 6,056 proteins in serum samples from 1,530 participants in the 100,000 Genomes Project with rare diseases.

Results: Technical performance was assessed by comparing SBI SomaScan v4.1 with Illumina Protein Prep in 50 samples, yielding a median Spearman r^2 of 0.58. SOMAmer probes exceeded the limit of detection (LoD) in >84% of all samples, >96% in 80% of samples, and >99% in 50% of samples (CLSI method). The median coefficient of variation was 7.7% (Q90 12.3%). Proteomics data effectively predicted gender (AUC = 0.986) and age ($r^2 = 0.934$), consistent with UK Biobank findings. Across 19 rare disease categories, 470 proteins were differentially abundant (FDR p-value <0.05). Categories with the highest differentially abundant proteins included congenital anomalies of the kidneys (n = 178), familial thoracic aortic aneurysm (n = 50), familial hypercholesterolaemia (n = 35), hypertrophic cardiomyopathy (n = 29), and dilated cardiomyopathy (n = 27). In contrast, intrauterine growth retardation, intellectual disability,

epileptic encephalopathy, and Brugada syndrome showed few or no differentially abundant proteins.

Conclusion: This study demonstrates the Illumina Protein Prep assay's high sensitivity and strong correlation with SBI SomaScan data. The abundant differential proteins in specific disease categories highlight the potential of proteomics to advance the diagnosis of certain rare genetic diseases by elucidating disease-associated genes and pathways.

C.O.I. Disclosure:

M. Brown: A. Employment (full or part-time); Modest; Genomics England. **A. Kousathanas:** A. Employment (full or part-time); Modest; Genomics England. **L. Hoa:** A. Employment (full or part-time); Modest; Genomics England. **G. Elgar:** A. Employment (full or part-time); Modest; Genomics England. **J. Ellingford:** A. Employment (full or part-time); Modest; Genomics England. **A. Ho:** A. Employment (full or part-time); Modest; Genomics England. **S. Walker:** A. Employment (full or part-time); Modest; Genomics England. **A. Crawford:** A. Employment (full or part-time); Significant; Illumina. **S. Freedman:** A. Employment (full or part-time); Significant; Illumina. **M. Mehan:** A. Employment (full or part-time); Significant; Illumina. **P. Prabhat:** A. Employment (full or part-time); Significant; Illumina. **A. Slatter:** A. Employment (full or part-time); Significant; Illumina. **N. Subramanian:** A. Employment (full or part-time); Significant; Illumina. **Y. Zhuang:** A. Employment (full or part-time); Significant; Illumina. **D. Daujotyte:** A. Employment (full or part-time); Significant; Illumina.

P23.004.B Comparative Clinical Utility of PacBio Long-read RNA Sequencing vs Short-read technologies in Diagnosing Rare Disorders

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Background: It is estimated that up to 33% of disease-causing variants disrupt splicing, but many are missed and are under-ascertained as these are not limited to canonical splice sites. As we integrate short-read RNAseq into clinical practice, it is essential to assess potential benefits of long-read sequencing (LRS) in diagnostics. This work assesses the clinical utility of PacBio long-read RNA sequencing for detecting aberrant splicing in rare disorders.

Material and Methods: Participants with Mendelian disorders harbouring variants likely affecting splicing were recruited across the UK and the Netherlands. RNA sequencing from 22 blood samples and four fibroblast cell lines were performed following the Kinnex full-length RNA protocol. HiFi reads from the Revio instrument were processed using Read Segmentation and Iso-Seq workflow.

Results: On average samples had 11–13 million full length non-chimeric reads. Pickup rate for OMIM genes was approximately 60–65% and 63%–70% for PanelApp genes, with fibroblast samples identifying a larger number of transcripts. Novel transcripts not in catalogue accounted for approximately 14% of total transcripts in both tissues, with this proportion increasing following cycloheximide treatment in fibroblasts. This study highlights cases where LRS was key in uncovering additional information and resolving events that were previously missed.

Conclusion: Although LRS may not be suitable for all patients referred for RNA testing, it successfully captured events missed by short-reads such as intron retention, skipping of multiple exons, differential transcript usage and phasing of variants serving a as valuable complement to short-read sequencing in a clinical setting.

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P23.005.C Nanowire-assisted, single-molecule sequencing by synthesis

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Background: Phasing errors limit sequencing-by-synthesis (SBS) to read lengths of 150–200 base pairs (bp) before error rates compromise single-nucleotide resolution. Single-molecule SBS circumvents this limitation by eliminating the need for cluster amplification, which is a primary source of phasing errors. By overcoming this issue, read lengths of up to 2000 bp become feasible with current SBS chemistry.

Materials and Methods: We have adapted Aligned Bio's silicon nanowire-based sensor platform to enhance fluorescence detection in SBS sequencing. By integrating this technology with standard four-color SBS chemistry, we enable high-sensitivity, single-molecule detection across large fields of view with reduced imaging time. A modified flow cell was designed to optimize this process and ensure compatibility with existing sequencing platforms, including the Illumina MiSeq.

Results: Using nanowire-enhanced fluorescence imaging, we successfully demonstrated single-molecule SBS sequencing with standard chemistry. This approach allows for high-throughput, long-read sequencing without compromising accuracy. Furthermore, we validated the adaptability of this system for use in industry-standard DNA sequencers.

Conclusion: Nanowire-enhanced single-molecule sensing enables long-read SBS sequencing using conventional four-color chemistry. This technology offers a cost-effective, plug-and-play solution for upgrading existing SBS-based sequencing instruments into long-read platforms. By improving efficiency and reducing sequencing costs, nanowire-assisted single-molecule SBS has the potential to significantly advance next-generation sequencing applications.

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P23.006.D Discovery of a Methylation Selective Cytidine Deaminase (mSCD) with potential for direct human methylome and genome sequencing

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5-Methylcytosine (5mC) is a key epigenetic marker involved in critical biological processes such as gene regulation, chromatin remodeling, and cell differentiation. Original methylation sequencing methods rely on bisulfite conversion, which introduces errors due to incomplete conversion and DNA degradation. These limitations have driven efforts to develop alternative approaches that preserve native DNA sequences while enabling accurate, high-throughput methylation detection. The use of a deaminase with specificity for 5mC over cytosine could revolutionize the field.

In this study, we employed a phenotype-to-genotype association approach directly on the phage fraction of a microbiome to uncover a previously uncharacterized family of deoxycytidine deaminases with methylation-selective cytidine deamination (mSCD) activity. We apply one such mSCD enzyme to human genomic DNA, followed by high-throughput short-read sequencing using Illumina and achieved high sensitivity detection of 5mC. Since mSCD directly converts 5mC, we achieve simultaneous genome and methylome sequencing. Applied to human brain samples, we demonstrate high sensitivity and specificity for methylation detection at CpG and CpA sites, expanding notably our understanding of non-CpG methylation where traditional methods fall short. Furthermore, pre-treatment with an enzyme that protects 5-hydroxymethylcytosine (5hmC), enables precise discrimination between 5mC and 5hmC.

This novel family of enzymes has the potential for broad applications, notably in the accurate genome and methylome sequencing across diverse sample types, including bacterial genomes, human tissues, and low-input materials such as cell-free DNA.

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P23.007.A Resolving complex regions with a novel sequencing technology delivering long-range insights

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Background: Short-read genome sequencing is a powerful tool in genetic diagnostics, but it faces challenges in phasing distant variants, detecting repeat expansions, identifying complex structural variants or resolving some highly complex regions. To overcome these limitations, we applied a novel sequencing technology from Illumina, which combines on-flow-cell library preparation with proximity-based long-range information, to sequence 42 challenging samples, previously diagnosed with different techniques including long-read sequencing.

Materials and Methods: DNA was extracted from 42 EDTA blood samples using various methods, resulting in diverse DNA quality profiles. Sequencing was performed using Illumina's Constellation Mapped Read technology. Samples were chosen based on their relevance to specific genomic challenges, including distant pathogenic variants in a single gene, complex loci (e.g., *CYP21A2*), structural rearrangements, repeat expansions (e.g., *RFC1*, *FGF14*), and transposon insertions.

Results: Preliminary results show that the technology phased variants in 9 of 11 cases where short-read genome sequencing failed. In the remaining two, extensive homology regions were present between the potentially pathogenic variants. Notably, in the *EYS* gene, two variants 389 kb apart were phased, despite a medium fragment size of less than 20,000 bp. Repeat expansions were correctly identified in all three cases. The proportion of fully phased genes varied with DNA quality.

Conclusion: This novel sequencing technology demonstrates significant potential in resolving challenging genomic regions, including phasing distant variants. By leveraging proximity-based long-range information, it bridges the gap between short and long read technologies, while maintaining workflow robustness and minimizing DNA quality requirements. Further analysis will clarify its diagnostic potential.

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P23.008.B Demonstrating the potential to increase diagnostic yield and equity of test provision in rapid genome testing for acutely unwell children through Constellation read technology

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Background: The Exeter Laboratory has delivered the NHSE nationally-commissioned rapid sequencing service for acutely unwell children since 2019. Results have been returned to >5000 families (current diagnostic yield 43%). The service uses short-read whole genome sequencing (NovaSeq™ X). Gene-agnostic hypothesis free analysis is undertaken in parent-child trios, assuming a fully penetrant disorder. Proband only and parent-child duo testing is limited to phenotype driven gene panel analysis, leading to inequity in test provision for this group.

Material and Methods: A proof of principle study was undertaken to assess the potential of Illumina's novel

Constellation read technology to increase diagnostic yield. Twenty-eight clinically challenging cases (41 samples) were analysed, representing variable family structures, DNA quality and extraction methods. Constellation enables a simplified on-flow-cell library prep (NovaSeq™ X) and combines accurate standard clustering and SBS sequencing with long-distance cluster proximity information through DRAGEN algorithms.

Results: Proband/duo phasing was demonstrated genome wide, accurately assigning clinically relevant variants to the correct haplotype in 18/18 cases, and de-novo haplotype-of-origin identified in 5/5 cases. Constellation correctly called 2/2 variants in difficult-to-map regions intractable to standard of care WGS and enabled full characterisation of 2/3 complex structural variants (SVs).

Conclusion: Early evaluation of Constellation has shown improved mapping and variant calling in complex genomic regions, extensive genome phasing, and detection of large (>50 bp) SVs. The potential to combine the accuracy of short read WGS with ultra-long phase blocks may enable gene agnostic testing for proband and duos and accurate recurrence risk counselling informed by de-novo haplotype-of-origin data.

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P23.009.C Mapping the spatial microbiome-host interactions in*Mycobacterium tuberculosis*(MTB) infected mouse lung with Stereo-seq FFPE

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Background: MTB causes tuberculosis by surviving within innate immune cells. However, the study of interactions between the host immune system and MTB is limited. Stereo-seq FFPE makes the groundbreaking advancement by enabling simultaneous capture of host spatial transcripts and mapping of microbiome on one FFPE tissue section. Here, we apply the Stereo-seq FFPE on MTB-infected mouse lung to provide a spatial perspective on microbiome-host interactions.

Material and Methods: C57BL/6 mice were infected with MTB and were housed in an animal BSL-3. Lung tissues were collected after 4 weeks of infection for spatial RNA profiles with Stereo-seq FFPE. MTB^+ lesion were confirmed by acidic dye in adjacent sections. All analysis was performed at resolution of bin 50 (25×25 um).

Results: Compared to uninfected control mouse, 4w infected mouses revealed the distinguished cell composition with higher percentage of alveolar macrophages (AMs), B cells, T cells and monocytes, but with reduced dendritic cells (DCs). We isolated spatial gene expression of AMs and DCs within 100 μm of MTB-infected cells and analyzed the differentially expressed gene profiles when compared to corresponding cell types in control. Enrichment of NOD-like receptor signaling and Toll-like receptor signaling indicated the activities of recognition of MTB. Upregulation of NF-kappa B signaling suggested the activation of macrophages and the release of various cytokines. Above biological response to MTB have been reported.

Conclusion: Stereo-seq possesses the ability to explore dynamic interactions between host and microbiome by providing simultaneous insights from both host and microbial communities at molecular and cellular level.

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P23.010.D New approach for molecular diagnosis of imprinting disorders: ONT adaptive sampling for targeted methylation analysis in Beckwith-Wiedemann syndrome

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The molecular pathogenesis of Beckwith-Wiedemann Syndrome (BWS) involves imprinting dysregulation of two regions, IC1 and IC2, located on 11p15.5. The telomeric IC1 domain, containing lncRNA H19 and IGF2, is completely methylated in paternally derived allele and unmethylated in maternally derived allele. The centromeric IC2, that includes KCNQ1, regulatory lncRNA KCNQ1OT1, and CDKN1C, exhibits the opposite methylation pattern. The loss of methylation at IC2 is observed in 50% of BWS patients. Hence, detection of abnormal methylation pattern at 11p15.5 is essential for diagnosis. Traditionally, bisulfite treatment and methylation-sensitive restriction enzyme treatment or methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) have been used.Here, we applied Nanopore adaptive sampling approach to perform targeted analysis of the methylation status within imprinted regions, achieving molecular diagnosis of BWS.Libraries were prepared using Ligation Sequencing Kit V14 and loaded onto a flow cell R10.4.1 on PromethION2 Solo running MinKNOW software. 23 genomic regions, subject to medically significant parent-of-origin-specific methylation, were selected as targets in a BED file. Next, base-calling was performed using Dorado from Epi2Me and reads were then aligned to GRCh38 using Minimap2, generating BAM files. Base modifications were detected using Modkit and visualized in IGV, which allowed the analysis of methylation patterns among selected regions. From the comparison with healthy controls' methylation profiles, we detected an hypomethylation of IC2, involving KCNQ1OT1:TSS in one patient.Hence, the described approach allows simultaneous genome-wide assessments of methylation status and represents a

very efficient one-step method applicable to the diagnosis of imprinting disorders other than BWS as well.

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P23.011.A Streamlined hybridization capture workflow for targeted long-read sequencing

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Background: Long-read sequencing has revolutionized the study of RNA isoforms, offering unparalleled insights into the complexity of gene expression. However, cost-effective detection of rare transcripts remains a challenge. Hybrid capture can reduce sequencing costs, detect rare isoforms, and simplify analysis. Here, we combine IDT hybridization capture with PacBio full-length RNA sequencing for deep isoform characterization.

Material and Methods: We sequenced six replicates from human brain samples and a universal human reference RNA (UHRR) standard. Three replicates underwent hybridization capture, while the other three were retained as whole transcriptome samples. The hybrid capture was performed using the new xGen Hyb and Wash Kit v3 along with a custom panel targeting 29 genes associated with neurological diseases. All samples were subsequently pooled into a 12-plex Kinnex full-length RNA library sequenced on one Revio and one Vega SMRT Cell, respectively.

Results: We obtained 59.7M and 36.2M segmented reads (S-reads) evenly distributed amongst the replicates, with consistent on-target rates of 85–86% for the targeted capture data, compared to 1.2–1.3% for whole transcriptome. Targeted enrichment detected all 29 targeted genes and ~250 total known isoforms, while whole transcriptome failed to detect 7 of them and only detected ~100 total known isoforms. Transcript abundances were preserved through the hybrid capture, and replicates showed high reproducibility.

Conclusion: PacBio long-read sequencing combined with the new streamlined IDT hybridization capture workflow can provide deep coverage and discovery of known and novel isoforms of target genes in a cost-effective manner.

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P23.012.B Two site comparison of a single PCR enrichment and nanopore sequencing workflow for complex clinically-relevant variants in seven genes associated with hereditary diseases

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Background: Variants associated with severe genetic disorders typically require multiple workflows beyond NGS for resolution in diagnostic and screening applications. Complex pathogenic variants involving short tandem repeats, copy number/structural variation (CNV/SV), and differentiating from genetically similar pseudogenes confound NGS. At two independent sites we evaluated the AmplideX Nanopore Carrier Plus Kit to genotype 7 challenging targets (*SMN1/2, FMR1, HBA1/2, HBB, GBA*) using a combination of PCR enrichment, nanopore sequencing, and analysis software in a novel and unified workflow.

Methods: We tested 98 residual clinical samples that had previously been genotyped by multiple methods. Target regions of gDNA were enriched in 4 PCR reactions, barcoded, pooled, and sequenced on MinION™ flow cells (R10.4.1) with a GridION™ (Oxford Nanopore Technologies) at two different sites. AmplideX Reporter software automated analysis and variant classification. Results were compared across sites to those obtained from multiple orthogonal.

Results: Across all samples, of the >95% that passed QC, ≥95% concordant with orthogonal results and across sites for all variant types. *SMN1/2* CNV (0 to > = 3) demonstrated 97% agreement on 33 samples. *FMR1* was 100% concordant for categorical match on 11 samples. SNVs/indel/SV concordance was >98%, observed in *SMN1/2*, *HBA1/2* and *HBB* (N = 40), and *GBA* (N = 14).

Conclusion: Our results highlight the reproducibility and accuracy of nanopore sequencing and dedicated bioinformatics software to consolidate workflows and automate both the analysis and interpretation of challenging variants relevant to hereditary genetic disease. These technological advances have the potential simplify variant detection within molecular labs.

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V. Guida: None. **A. Morella:** None. **B.J. Killinger:** A. Employment (full or part-time); Significant; Asuragen. **T. Markulin:** A. Employment (full or part-time); Significant; Asuragen. **S. Borel:** A. Employment (full or part-time); Significant; Asuragen. **M.C. D'Asdia:** None. **N. Grifone:** None. **A. Onori:** None. **M.R. D'Apice:** None. **L. Pallante:** None. **J.A. Baker:** A. Employment (full or part-time); Significant; Asuragen. **I. Wolf:** A. Employment (full or part-time); Significant; Asuragen. **J. Turner:** A. Employment (full or part-time); Significant; Asuragen. **J. Janovsky:** A. Employment (full or part-time); Significant; Asuragen. **C. Parker:** A. Employment (full or part-time); Significant; Asuragen. **C. Edwards:** A. Employment (full or part-time); Significant; Asuragen. **S. Filipovic-Sadic:** A. Employment (full or part-time); Significant; Asuragen. **B. Hall:** A. Employment (full or part-time); Significant; Asuragen. **A. De Luca:** None.

P23.013.C EpiPlex - A Platform for Multi-Target Detection of RNA Modifications

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Background: RNA is subject to chemical modifications that collectively represent the epitranscriptome. The most frequent modifications in human mRNA are m6A, inosine and pseudouridine. By impacting RNA splicing, stability, folding, trafficking and translation, the epitranscriptome plays an important role in the posttranscriptional control of gene expression. Dysregulation of RNA modifications has been associated with multiple diseases, making them potential targets for therapeutic interventions. Despite their important role in gene regulation, reliable commercial methods for the detection of RNA modifications are still lacking.

Material and Methods: Here we present EpiPlex RNA modification encoding, a proximity barcoding assay with sequencing readout that enables multi-target detection in a simple 1-day library prep workflow, providing the location and relative abundance of RNA modifications. The EpiPlex works with RNA-limited sample types and even with FFPE RNA. To make data analysis accessible to all scientists, we have developed EpiScout, a custom-tailored end-to-end analysis pipeline with a machine learning trained peak caller.

Results: The current version of EpiPlex detects m6A and inosine, with extendability to other modifications. In addition to providing a technological overview, we will present biological data showing how the function of the exon junction complex shapes the transcriptome-wide distribution of m6A.

Conclusion: Taken together, the EpiPlex™ platform is a user-friendly tool that enables basic research and novel applications in diagnostics and RNA therapeutics.

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P23.014.D Performance evaluation of accelerated Next Generation Sequence (NGS) analysis pipelines

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Background: Next-generation sequencing (NGS) analysis pipelines are routinely used in current clinical and research settings. However, their CPU-based NGS data processing approach has led to extended turnaround times, which affect the scaling of whole-genome sequence (WGS)-based clinical analysis and population-scale genomics studies. Two leading accelerated genomic analysis platforms - DRAGEN and Parabricks address this challenge by optimizing NGS-data processing on Field-Programmable Gate Arrays (FPGA) and Graphical Processing Units (GPUs). We present a side-by-side comparison of these platforms, addressing a clear gap in recent literature where most studies focus on evaluating single platforms in isolation.

Material and Methods: We implemented accelerated (DRAGEN and Parabricks) and CPU-only pipelines following vendor-specific and best-practices guidelines. They were then evaluated focussing on runtime, speedup, coverage-based scalability and variant calling performances. Here, Parabricks pipeline was assessed across L4, A100 and H100 GPUs.

Results: Both accelerated pipelines demonstrated shorter runtimes compared to CPU-only approaches, with Parabricks-H100 demonstrating the fastest performance, followed by DRAGEN. In mapping, DRAGEN outperformed Parabricks (L4 and A100) and matched H100 speedups. During variant-calling, Parabricks (A100 and H100) demonstrated higher speedups than DRAGEN. In coverage-based-scalability, DRAGEN and Parabricks-H100 mapping showed positive trends, while other configurations failed to scale effectively. Moreover, all variant-callers achieved high scores in both performance and concordance analysis. Furthermore, our optimized Parabricks variant-calling pipeline (developed based on GPU-profiles) demonstrated potential for further speedup enhancements.

Conclusions: We publicly released our NGS-pipelines (MIT License) and offer valuable implementation insights to address time-sensitive and scalability challenges in current clinical genomics and research settings.

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P23.015.A Verification of an integrated amplification-based nanopore sequencing assay and automated software solution to genotype challenging, clinically relevant variants

Connor Parker, Juliette A. Baker, Walairat L. Wolf, Jon Kemppainen, Justin Janovsky, Jonathan Turner, Bryan J. Killinger, Cody Edwards, Monica Roberts, Theodore Markulin, Eduardo Priego, Mahesh Yarasi, Mia K. Mihailovic, Brian C. Haynes, Bradley Hall, John N. Milligan

Asuragen, a Bio-Techne brand, AUSTIN, TX, USA.

Background: Accurate resolution of variants correlated with severe genetic disorders is crucial for diagnostic and screening applications. While NGS is used broadly, it is frequently incapable of resolving complex pathogenic variants involving short tandem repeats, copy number/structural variations (CNV/SV), and pseudogenes. Here, we present verification of a method combining AmplideX PCR enrichment, nanopore sequencing, and analysis software to genotype 11 high-prevalence and challenging targets (*CFTR, SMN1/2, FMR1, HBA1/2, HBB, F8* inversions*, GBA, CYP21A2/TNXB*).

Material and Methods: We tested >425 unique samples to verify accuracy, precision, sensitivity, and specificity. Target regions were enriched with 4 PCR reactions, barcoded, pooled, and sequenced in a single assay workflow. Sequencing was performed on MinION™ flow cells (R10.4.1) with a Mk1B or GridION™ instrument (Oxford Nanopore Technologies). We developed software to automate analysis/reporting and compared results to multiple orthogonal methods.

Results: Across all variant types, verification demonstrated ≥95% concordance with orthogonal results. SNVs/indel concordance was >98% across all genes. *SMN1/2* CNV (0 to >=3) showed ≥96% agreement. *FMR1* was ≥95% concordant for repeat sizing and AGG interrupts. *GBA* and *CYP21A2/TNXB* were differentiated from their pseudogenes, enabling resolution of fusions and SVs and correct phasing of SNVs/indels. *F8* intron inversions showed 100% agreement.

Conclusion: Our results demonstrate the utility of combining nanopore sequencing with novel PCR chemistry and algorithms to resolve clinically relevant genotypes insurmountable by routine NGS using affordable, scalable technologies in a single workflow suitable for most laboratories. The software enables custom variant reporting, ClinVar classification, and in-silico novel variant predictions to streamline interpretation.

C.O.I. Disclosure:

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P23.016.B Constellation mapped read technology, a novel on flow cell library prep for both high quality short reads and proximity information in one prep delivering an expanded genome

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Background: High quality short read whole genome sequencing (WGS) delivers exceptional quality throughout most of the genome, but still struggles with more challenging regions and variant types. These challenges include variant calling in difficult-to-map regions such as pseudogenes and segmental duplications, calling structural variants, and variant phasing.

Material and Methods: Constellation technology, a simplified on-flow cell library preparation method on the NovaSeq™ X delivers an expanded genome by enhancing short read sequencing data with proximity information. Proximity information is leveraged to recover those genomic regions and variant types that are especially challenging in standard short read WGS delivering an expanded genome.

Results: To determine performance of this novel on flow cell method, the proximity analysis has been demonstrated on a range of cell lines and blood samples with a range of DNA qualities. We present improvements in small variant calling performance across the genome and highlight specific examples in difficult-to-map medically relevant genes. We also describe improvements to phasing haplotypes, enabling >98% of genes to be fully phased in a standard GM24385 (HG002) cell line sample and demonstrate how fully phased genes resolve compound heterozygotes. Finally, we describe novel methods to enable the detection of large variants, repeat expansions and complex rearrangements with the proximity mapping of short reads. We demonstrate improvements across the genome with specific examples and previously characterized balanced structural variants.

Conclusion: Constellation mapped read technology delivers an expanded genome by enhancing short read sequencing data with proximity information.

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P23.017.C Redefining nanopore sequencing with Duplex Sequencing by Expansion (SBX-D): A high-accuracy, high-throughput sequencing technology

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Background: Nucleic acids are small, informatically dense molecules. Single molecule sequencing of nucleic acids is limited by the spatial differentiation of neighboring nucleotides.

Material and Methods: Here we report on Sequencing By Expansion (SBX) which solves these issues by creating an Xpandomer (Xp) surrogate molecule with >50x separation between high signal-to-noise nucleotide reporters. This voltage controllable structure produces discrete electrical signals when measured across a biological pore. Xpandomers are generated in an enzymatic, template-directed manner using a mutated Y-family DNA polymerase (Xp Synthase), expandable nucleotide triphosphates (XNTPs), and a polymerase enhancing moiety (PEM). Xp synthesis is separate from the measurement, with an on-support automated workflow. Specialized components of the Xp structure

are designed to maximize capture rate and translocation efficiency through the pore, which allows parallelized measurement on a 8M sensor array. We demonstrate the capability of this technology with Sequencing By Expansion Duplex (SBX-D) which links both strands of the target DNA in a single sequencing read. This approach uses intramolecular consensus of the complementary reads to identify discordant calls and achieve high accuracy.

Results: With 1 hour of sequencing we show >34x coverage of each individual in a 7-plex genome-in-a-bottle library, with observed duplex concordant base quality >Q39 and F1 scores of >99.6% (SNV) and >99.2% (InDel) using our SBX Variant Caller.

Conclusion: SBX is a transformative sequencing technology that pairs high throughput and high accuracy to improve existing applications and to enable innovation in biological research and molecular diagnostics.

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P23.019.A A streamlined and modular workflow tuned to work across multiple sequencing platforms provides improved capture performance for intact gDNA, total RNA as well as low input FFPE

Sangwon Lee[1], *Sergey Shiryaev*[2], *Keith Chen*[2], *Brandyn Clark*[2], *Bilan Hsue*[2], *Nikki Liu*[1], *Adam Janssen*[2], *Jeff Fox*[2], *Nedda Saremi*[1], *Inlora Jingga*[1], *Kyeong-soo Jeong*[1], *Khine Win*[1], *Gilbert Amparo*[1], *Neelima Mehendale*[1], *Jayati Ghosh*[1], *Cindy Hon*[1], *Bahram Arezi*[2]

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Background: The application of NGS in cancer may be affected by limited availability and quality of DNA and RNA, as well as the varying tumor fraction in samples. Additionally, practical factors such as workflow turnaround time, ease of use and overall assay performance play crucial roles in determining whether NGS is the preferred method for detecting mutations.

Material and Methods: Various sample types, including intact, FFPE, and cfDNA ranging in input from 1–500 ng were used. Modular workflows were utilized for library preparation/enrichment, and sequencing was performed on three platforms including Illumina Novaseq, Element Aviti, and MGI DNBSEQ G400.

Results: Library prep using 3 bacteria of varying GC content (30%–67%) shows high coverage uniformity and minimal bias (Fold80 of ~1.1). Slight modifications in bait hybridization conditions counteract the sequencer-specific AT/GC bias (e.g., Illumina *vs*. MGI) and improve coverage uniformity. Our novel fast hybridization buffer provides maximum complexity (5–7% duplicates at 6Gb for 8 pooled exome V8 libraries) while maintaining high uniformity (Fold80 of 1.4–1.57) and coverage (93–96% at 30X base coverage) across 3 sequencing platforms, specifically to address the issue of complexity loss with high multiplexing target enrichment.

Conclusion: We present a modular workflow, optimized for a wide range of sample types and inputs, standard or molecular-barcoded adaptors, fast or overnight bait hybridization, and 384UDI optimal for multiple sequencing platforms (Illumina, Element, and MGI). With small changes in the protocol, efficient long read capture (>5 kb) and sequencing can be accomplished. For Research Use Only. Not for Use in Diagnostic Procedures. PR#7001-3721

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P23.020.B Synthetic DNA spike-ins to track samples for NGS sequencing

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Background: The demand for NGS processing has grown due to advancements in library preparation and sequencing technologies, as well as reduced sample processing costs. NGS platforms have broadened the scope of genomics research, enabling studies on rare genetic diseases, cancer genomics, microbiome analysis, infectious diseases, and population genetics. As the number of samples increases, the risk of mislabeling during extraction and processing also rises.

Materials and Methods: We propose synthetic DNA spike-ins (SDSIs) for sample tracking, using a uniquely identifiable core DNA sequence approximately 160 base pairs (bps) long. Each SDSI core consists of 96 distinct DNA sequences from rare Archaea genomes, with no significant homology to Homo sapiens or viral genomes, preventing false detection or cross-identification.

Results: We tested two SDSIs across various library preparation technologies, including bead-linked Tn5, free Tn5, and enzymatic fragmentation, to evaluate their usability in a lab setting. We also assessed the optimal concentration for bioinformatic detection and the potential for contamination. Additionally, nucleic acid extraction from buccal swabs was tested incorporating SDSI.

Conclusion: The goal is to provide a reliable tool for unequivocally identifying samples before sequencing, combining this method with traditional sequencing indices to ensure high-precision results.

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E. Guidi: None. **C. Balestrieri:** None. **G. Tonon:** None. **D. Lazarevic:** None.

P23.021.C Increased library conversion rate enables detection of low-frequency variants

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Background: Next-generation sequencing (NGS) methods have enabled a broad set of clinical applications. The cost and throughput advantages of NGS may be offset by poor base accuracy and conversion rates. With technologies reporting accuracy of Q40 and beyond, the upstream and downstream steps of the sequencing process provide opportunities for

optimization. Library conversion rate is important for the identification of rare genomic variants.

Material and Methods: We have evaluated four library preparation kits: DNA Library Prep (Watchmaker Genomics), KAPA EvoPrep (Roche), cfDNA Library Preparation (Twist Bioscience), and Illumina Cell-Free DNA Prep (Illumina). We benchmarked these against KAPA HyperPrep (Roche). A total of 30 ng of cfDNA was used for library preparation, followed by target enrichment with Twist Standard Hybridization Reagent Kit using a 118 kbp panel. KAPA EvoPlus (Roche) and DNA Library Prep (Watchmaker Genomics) will also be evaluated on genomic DNA and DNA from FFPE.

Results: KAPA EvoPrep, cfDNA Library Preparation, and DNA Library Prep kits showed a 7–10% and 12–15% increased conversion rate compared to KAPA HyperPrep, with overnight ligation and 15-minute, respectively. The conversion rate of the Cell-Free DNA Prep kit did not surpass the performance of the HyperPrep kit, and exhibited an increased GC dropout in GC rich regions associated with a significant drop in coverage.

Conclusion: A high conversion rate and even coverage over the targeted regions enable the detection of rare variants. When combined with a sequencing instrument with high accuracy, ultra-rare variants may be detected.

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P23.022.D VarMeter2, a new and improved programme for predicting the effect of genome missense variants

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Background: In comprehensive genome analysis, data on the predicted pathogenicity of missense variants is one of the most useful pieces of information. However, there are cases where currently available prediction programs (such as SIFT, PolyPhen-2, and CADD) are insufficient. Therefore, there is a need for the development of novel programs with high accuracy. Recently, we developed a program called 'VarMeter' based on a new inference method based on protein 3D structural changes [ref1]. In this study, we evaluated the broader applicability of VarMeter and made improvements to improve its prediction accuracy.

Material and Methods: We compared and learned structural features such as nSASA, mutation energy, and predicted local distance difference test (pLDDT) scores for 296 pathogenic variants and 240 benign variants extracted from the ClinVar database, and constructed an improved variant prediction program, VarMeter2. We used VarMeter2 to verify the prediction accuracy for the validation ClinVar dataset and missense variants of N-sulfoglucosamine sulfohydrolase (SGSH), an enzyme related to Sanfillippo syndrome A hydrolase (SGSH) missense variants using VarMeter2. We measured the enzymatic activity of the novel SGSH variants we identified and confirmed them.

Results: The prediction accuracy of the ClinVar dataset and the SGSH missense variant dataset was 82% and 84%, respectively. The enzyme activity of the novel SGSH variants that were predicted to be pathogenic was lacking.

Conclusion: VarMeter2 is one of the useful prediction programs for evaluating missense variants in comprehensive genomic analysis of rare diseases.

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P23.023.A Exploring the feasibility of droplet digital PCR to improve turnaround times and improve patient outcomes in the NHS

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Background: The demand for rapid genomic testing is rising with the introduction of personalised therapies and updated risk stratification guidelines. Many solid cancer pathways use NGS, a valuable technique that generates large quantities of data but often results in lengthy analysis times, high costs and a low proportion of actionable results. To achieve optimal patient outcomes, some treatment is required to be administered within days of specimen collection and current pathways often struggle to achieve this. For endometrial cancers, *POLE* mutational status has become integral to risk stratification and for melanoma, *BRAF* V600 mutational status is important for treatment options. Droplet digital PCR (ddPCR) offers the potential to provide rapid testing. This study aims to evaluate ddPCR for rapid *BRAF* testing in melanoma and *POLE* testing in endometrial cancer.

Material and Methods: Clinical samples, previously tested for *BRAF* and *POLE* mutations, were reanalysed using commercially available ddPCR kits. Key performance metrics, including turn-around time (TAT), assay concordance, sensitivity, and specificity, were evaluated. Additionally, workflow efficiency and scalability were analysed to determine the suitability of ddPCR for adoption within an NHS laboratory.

Results: For melanoma samples, *BRAF* testing detected mutations with 100% concordance and showed potential to reduce TAT by ~17 days. A positive test would allow for patients to begin targeted therapy sooner and a negative test can prioritise patients for further testing. Final results, including *POLE* data, will be presented at the conference.

Conclusion: ddPCR is a promising technology to provide rapid testing for patients with solid cancers.

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P23.024.B Reduced transcriptional output and allelic biases from the pathogenic *SNCA* p.A53T missense variant, with implications for Parkinson's disease pathology

Hannah Macpherson[1], *Emil Gustavsson*[1], *James Evans*[1,2], *Claire Anderson*[1], *Kylie Montgomery*[1], *Jasmaine Lee*[1], *Henry Houlden*[1], *John Hardy*[1], *Nicholas Wood*[1], *Sonia Gandhi*[1,2], *Mina Ryten*[3,1]

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Disease-associated genetic variants are often assumed to operate through a single primary mechanism across tissues, but the reality

is likely more complex. Determining the impact of genetic variants based solely on gene structure can be challenging. Since missense variants can also affect splicing, this could impact on our understanding of disease mechanism with the potential for the development of flawed therapeutic strategies. *SNCA*, a gene linked to Parkinson's disease (PD), exemplifies this complexity, as it harbours both missense variants and copy number variants (CNVs). While current antisense oligonucleotide therapies aim to downregulate *SNCA* expression, dosage may not be the sole mechanism by which missense variants and CNVs operate to cause PD. Here we used long-read RNA sequencing to explore the impact of *SNCA* c.209G>A (p.A53T) on *SNCA* expression and splicing in iPSC-derived midbrain dopaminergic neurons, the cell type progressively lost in PD. We developed an allele-specific expression (ASE) analysis pipeline which allowed us to define underlying transcript structures and identify how transcription from the mutant allele was affected. Our analysis revealed both significantly fewer transcripts from the mutant allele, and changes in transcript proportions. Cycloheximide treatment further showed that this reduction was partially due to nonsense-mediated decay.These findings raise important questions about variant interpretation and pathogenesis, with potential implications for RNA-based therapeutic strategies targeting *SNCA*. We anticipate that this effect is not unique to *SNCA* and suggest that similar allele-specific transcript proportion changes may occur in response to missense variants at other neurodegenerative disease loci.

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P23.025.C PegRNA optimization for F508del correction: identifying key design features for AT-rich regions

Olga Volodina[1], *Anna Demchenko*[1], *Arina Anuchina*[1], *Oxana Ryzhkova*[1], *Valeriia Kovalskaia*[1], *Ekaterina Kondrateva*[1], *Elena Amelina*[2], *Vyacheslav Tabakov*[1], *Alexander Lavrov*[1], *Svetlana Smirnikhina*[1]

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Background: We chose prime editing (PE) to develop causal treatment for patients with F508del variant in the *CFTR* gene. To reach greater efficiency of PE it is important to rationally design variable parts of guide RNA (pegRNA), such as primer binding site (PBS), reverse transcription template (RTT), and spacer. However, current guidelines are inadequate for genomic regions with GC content below 40%, a characteristic of the *CFTR* gene locus harboring the F508del variant, so it is important not only to identify the most effective pegRNA but also to understand key patterns in the selection of its variable regions.

Material and Methods: 24 plasmids with different pegRNA sequences were designed and assembled. Two patients' airway basal cells with the homozygous F508del variant in the *CFTR* gene were used in experiments with delivery of genetic constructions by non-viral methods. Editing efficiency was assessed after 72 hours using deep targeted sequencing.

Results: The best efficiency rates were detected in pegRNA19, 20, 21, 22, selected for PE2-NG tool, - 21%, 23.6%, 17.3%, 15.04%, respectively. After the data analysis, we noted that composition and length of the PBS mostly affected the efficiency - lower GC content needs a longer region, while a high GC content in the RTT only plays role when PBS is selected correctly.

Conclusion: We identified the most effective pegRNA sequences, gene correction rates were up to 24%. In addition, we managed to discover recurring motifs that may be relevant for the selection of optimal pegRNAs for the AT-rich genome regions.

C.O.I. Disclosure:

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P23.026.D Expanded content and improved performance of Illumina Protein Prep

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Background: Illumina Protein Prep (IPP) is a comprehensive high-throughput proteomics solution combining Illumina's next-generation sequencing with Standard BioTool's SOMAmer™ technology. This powerful approach utilizes SOMAmers (Slow Off-rate Modified Aptamers) to bind to proteins with high affinity. Here we present a newly updated version of IPP with an expanded menu of 9464 unique human proteins based on 11K SOMAmer panel and improved overall performance.

Material and Methods: To determine overall performance of the assay, 205 donor matched plasma and serum were split into various replicates across 24 automated runs and sequenced using NovaSeq™ systems. Comparisons per-protein were determined using 31 plasma samples for the new method compared to either the previous smaller IPP panel or the microarray readout. A Limit of detection (LoD) ratio (Q25 population/inferred LoD) was calculated using a healthy population of 200 samples.

Results: Total median precision of the assay was measured in replicates of 5 donors to be ~4.4% CV in Serum and ~5.5% CV in plasma with Q90 of ~7.1% and ~8.4% respectively. Median spearman correlation per SOMAmer were 0.92, 0.87, and 0.82 for array vs array, NGS vs NGS, and NGS vs array respectively. The percentage of protein targets that were above the LoD was ~92% in plasma and ~95% in serum.

Conclusion: This study shows that the new version of Illumina Protein Prep has good performance with high precision, high analytical sensitivity, and close agreement to the previously used microarray readout.

C.O.I. Disclosure:

A. Slatter: A. Employment (full or part-time); Significant; Illumina. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Illumina. **M. Dorwart:** A. Employment (full or part-time); Significant; Illumina. **M. Mehan:** A. Employment (full or part-time); Significant; Illumina. **S. Freedman:** A. Employment (full or part-time); Significant; Illumina. **I. Alves**

Ines: A. Employment (full or part-time); Significant; Illumina. **N. Subramanian:** A. Employment (full or part-time); Significant; Illumina. **D. Jones:** A. Employment (full or part-time); Significant; Illumina. **Y. Cao:** A. Employment (full or part-time); Significant; Illumina. **C. Randise-Hinchliff:** A. Employment (full or part-time); Significant; Illumina. **F. Kaper:** A. Employment (full or part-time); Significant; Illumina.

P23.027.A Tracking allogeneic NK cell (SAR445419) treatment by chimerism assay in patients with relapsed or refractory acute myeloid leukemia patients

Francesco Lo Presti[1], *Jacqueline Courta*[2], *Anna Regnault*[3], *Florence Gillot*[3], *Claire Mariet*[1], *Marie-Hélène Pascual*[4], *Agata Drewniak*[5]

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Background: SAR445419 (SAR'419) cells are an allogeneic, off-the-shelf, donor-derived, non-genetically modified NK cell-based investigational immunotherapy evaluated in the TED17749 Phase 1, dose escalation study in six participants with relapsed or refractory AML. Monitoring SAR'419 cell persistence was required to better correlate their presence to clinical outcomes but was challenging in clinical samples.

Material and Methods: Discriminatory power of Devyser chimerism assay, based on the allele frequency of 24 indels genetic markers, was validated with 3-genome combinations (from 10 Humans). Protocol adaptations were done to monitor SAR'419 persistence in participant's blood and bone marrow samples.

Results: SAR'419 and patient baseline profiles determined informative markers discriminating patient's cells from SAR'419 batches. The method detects as low as 0.05% of allogeneic cells in 3-genome mixed samples. Confident marker profiles with as low as 0.06 ng of DNA/µL permitted the profiling of lymphodepleted patients. An 8-week SAR'419 tracking from different donors revealed a maximum presence of up to 30% of the total PBMC two weeks after administration. Their detection in bone marrow aspirates confirmed their ability to migrate to diseased areas. Atypical marker frequencies allowed the detection of potential AML presence as well as previous stem cell transplant. Faced to missing baseline patient sample, we were able to establish a "virtual screening profile" by deconvoluting each 24-marker curves.

Conclusion: This NGS assay is a suitable method to monitor confidently the persistence of SAR'419 cells in patients in Phase1 studies and can potentially be used to track any allogeneic modified cells infused into patients.

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P23.028.B Reliable and comprehensive analysis of SNVs, indels and repeat expansions in ataxia-associated genes using long-read sequencing

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Background: Spinocerebellar Ataxias (SCAs) are a genetically heterogenous class of progressive movement disorders, caused by SNVs, indels, and most importantly repeat expansions, in dozens of genes. Repeat expansions cannot be identified reliably through NGS, and the use of gene-specific PCR assays results in incomplete and fixed diagnostics. New causative repeat expansions are identified regularly, marking the need for a comprehensive and flexible gene panel. Here, we introduce a one-test-fits-all approach for SCA diagnostics using long-read sequencing (LRS).

Material and Methods: DNA of 12 patients was sequenced using Nanopore's 'adaptive sampling' method (Oxford Nanopore Technologies) for a neurogenetics gene panel including 27 genes known to cause SCA by repeat expansion. Data was compared to diagnostic repeat expansion data of several genes and WES data. Data was processed and analyzed using the open-source MOLGENIS Variant Interpretation Pipeline (VIP) 7.9.0.

Results: Comparison between LRS and PCR data showed 20 out of 23 repeat lengths on 7 different ataxia-related genes in 12 patients were concordant on both alleles (max 2 repeat units difference). The non-concordant repeats differed by max 4 repeat units. Additionally, the repeat motif could be determined reliably. Comparison between LRS and NGS data in 6 samples showed all 28 NGS-called indels were also found by LRS, just as 198 out of 202 NGS-based SNVs. Subsequently, 10 patients were sequenced prospectively for analysis of ataxia-related repeat expansions.

Conclusion: LRS enables adaptive, reliable and comprehensive analysis of SNVs, indels and repeat expansions in ataxia-associated genes and has the potential to increase diagnostic yield.

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P23.030.D Rejuvenation and antimicrobial potential of Platelet-rich plasma (PRP) and its impact on the pathogenesis of selected diseases

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Background: Platelet-rich plasma (PRP) is a plasma fraction with a platelet concentration three to five times higher than baseline levels, known for activating cellular pathways that promote cell proliferation and regeneration. This study investigated PRP's dual potential: its rejuvenating effect on knee osteoarthritis and its antimicrobial properties against clinical microbial isolates.

Material and Methods: PRP's antimicrobial activity was assessed using microbial strains isolated from 100 patients with urinary and soft tissue infections. Biofilm-forming ability was identified using Tube test method and Real-Time PCR targeting the *ica* gene cluster. Changes in the metabolic activity following PRP treatment were analyzed through leucine aminopeptidase and aspartic proteinase enzyme assays and quantification of total protein content by Bradford assay. For osteoarthritis, isokinetic measurements were conducted to evaluate joint mobility, strength, and muscle endurance in patients after PRP therapy.

Results: PRP demonstrated significantly stronger antimicrobial effects compared to controls. PRP reduced aspartic proteinase activity and increased total protein content in all tested strains

except *E. faecalis*, which showed the lowest antimicrobial response after PRP treatment. Isokinetic evaluations revealed that PRP significantly enhanced joint strength, reduced pain, and improved muscle endurance in osteoarthritis patients.

Conclusion: Obtained results confirm the antimicrobial effect of PRP on clinical strains identified as strong biofilm formers, as well as its rejuvenating effect on patients with knee osteoarthritis. This study is the first to examine the impact of PRP on the metabolic activity of selected microbial isolates, demonstrating a reduction in aspartic proteinase activity and an increase in total protein content.

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T. Becirevic: None. **I. Eminovic:** None. **M. Ibrisimovic:** None. **N. Ibrisimovic-Mehmedinovic:** None.

P23.031.A Epigenomic Breakthroughs: Redefining Diagnostic Paradigms in Genomic Medicine

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Although cytogenomic investigation methodologies have achieved significant advancements, leading to increased sensitivity in the detection of genomic variations, the understanding of the clinical relevance of these variants has not progressed proportionally. Consequently, one of the major challenges in contemporary genomic medicine is the high number of variants of uncertain clinicalsignificance, as well as a substantial proportion of patients who remain undiagnosed even after investigation with the primary methodologies. DNA methylation assessment, through approaches such as "Epi-signatures," has demonstrated significant potential in addressing this challenge, offering a newdimension of investigation. In this study, we aimed to evaluate the diagnostic applicability of methylation profiling in 29 patients with genomic variants classified as VUS and/or likely pathogenic, using the Infinium MethylationEPIC assay. From the methylation data, we applied various analytical approaches, including ontological analyses and Epi-signatures, to confirm or complement moleculardiagnoses. Our study revealed clinically relevant epigenomic alterations and enabled the diagnosis or diagnostic complementation in 12 cases, including eight cases with potentially affected biological pathways correlated with the respectiveclinical phenotypes, and six cases with conclusive or moderate epi-signatures corresponding to clinical conditions investigated using the EpiSign tool. This study thus underscores the relevance of epigenomic investigation in cases lacking a definitive diagnosis through traditional genomic methodologies, demonstrating thatthis approach can operate at both extremes: aiding in the exclusion or confirmation of diagnostic suspicions associated with a genomic variant.

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P23.032.B Genome-wide detection of gross copy number alterations associated with genetic conditions using digitalMLPA

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Background: Fast and robust characterisation of gross copy number (CN) changes is essential for cytogenetic applications. Next generation sequencing (NGS)-based approaches and optical genome mapping (OGM), as well as karyotyping and FISH, remain relatively costly, labour-intensive, and require complex bioinformatics analysis, resulting in longer turnaround times. qPCR-based methods typically only target a limited number of regions. Thus, there is an urgent need for cost-effective genome-wide CN characterisation of cytogenetic samples in a high-throughput fashion. Here, we showcase genome-wide CN characterisation at a ~2-4 Mb resolution in a panel of cell lines harbouring clinically significant CN alterations (CNAs), using SALSA® digitalMLPA™ Probemix D024 (test version) consisting of ~1200 probes.

Material and Methods: D024 was used to analyse the Coriell CNV01 panel, comprising DNA samples from 43 cell lines harbouring a diverse range of chromosomal alterations. digitalMLPA-derived PCR amplicons were sequenced on a NextSeq 1000 Sequencing System (Illumina). FASTQ files were analysed using Coffalyser digitalMLPA™ (MRC Holland).

Results: The digitalMLPA assay, D024, showed a high concordance (>98 % for 51.084 datapoints) with CN data reported by the Coriell Institute for the CNV01 panel. Detected aberrations included gains and losses of whole chromosomes, as well as subchromosomal CNAs. Samples harbouring multiple CNAs were successfully characterised.

Conclusion: Using the SALSA® digitalMLPA™ Probemix D024, a wide range of gross CN alterations can be reliably determined genome-wide in a cost-effective manner, with a short (48–72h) turnaround time in one single reaction.

C.O.I. Disclosure:

S. Palit: A. Employment (full or part-time); Significant; MRC Holland. **B. Lamers:** A. Employment (full or part-time); Significant; MRC Holland. **A. Benard-Slagter:** A. Employment (full or part-time); Significant; MRC Holland. **J. Smout:** A. Employment (full or part-time); Significant; MRC Holland. **M. Clausen:** A. Employment (full or part-time); Significant; MRC Holland. **K. de Groot:** A. Employment (full or part-time); Significant; MRC Holland. **S. Savola:** A. Employment (full or part-time); Significant; MRC Holland. **L. Stolk:** A. Employment (full or part-time); Significant; MRC H.

P23.033.C digitalMLPA: a new frontier in the simultaneous determination of copy number and methylation status across multiple imprinting-associated differentially methylated regions

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Background: Multi-locus imprinting disturbances (MLIDs) involve aberrant methylation patterns at multiple loci, which complicates the diagnosis and management for a subset of imprinting disorder patients. To address the current difficulties in studying MLIDs, we developed a new SALSA® digitalMLPA™ probemix for methylation pattern detection that combines the reliability of methylation-specific SALSA® MLPA® (MS-MLPA) with the high coverage of NGS. This could provide a robust solution for simultaneous detection of methylation aberrations across multiple loci.

Material and Methods: The methylation-specific digitalMLPA probemix (test version) was developed for simultaneous detection of the copy number (CN) in 37 targets across most autosomes, and the methylation status in 27 imprinting-associated differentially methylated regions (iDMRs). 126 control samples and over 30 positive samples (blood and cell lines) were tested. A subset of positive samples was also tested with MS-MLPA. Additionally,

some iDMRs were studied on dried blood spot (DBS) card-derived DNA samples from healthy newborns.

Results: In control samples, the expected CNs were observed and most imprinting regions had median methylation percentages between 45–64%, except for two iDMRs (86% and 94%). A positive sample with known 11p15.5 methylation aberrations showed concordant results when tested with MS-MLPA and MS-digitalMLPA. Moreover, MS-digitalMLPA detected an additional aberrant iDMR. DBS sample data was robust, demonstrating the versatility of MS-digitalMLPA across different blood-derived sample types.

Conclusion: Our results demonstrate that MS-digitalMLPA, like MS-MLPA, can accurately determine both CN and methylation status. This study underscores the potential of MS-digitalMLPA for detecting methylation aberrations across multiple loci in a single assay.

C.O.I. Disclosure:

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P23.034.D Evaluating variants of uncertain significance in zebrafish: a proof of concept with a COL1A2 variant

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Background: Genomic sequencing has led to the identification of numerous variants of uncertain significance (VUS), posing challenges for clinical decision-making. While segregation studies and in silico prediction tools are helpful in VUS classification, they are not always feasible, emphasizing the need for functional testing to accurately interpret these variants.

Material and Methods: In this study, we investigated the pathogenicity of the *COL1A2* c.2123G>A VUS, identified in a 59-year-old female with recurrent fractures, using zebrafish as a model organism. Using prime editing, we previously introduced the VUS into the zebrafish genome, alongside a benign control and a known pathogenic variant.

Results: Comprehensive skeletal phenotyping revealed no significant abnormalities in the VUS zebrafish model while the positive control showed an increased number of fusions, compressions, fractures, more notochord and intervertebral ligament mineralization and more scoliosis of the vertebral column, suggesting the variant is likely benign.

Conclusion: Our findings highlight the effectiveness of zebrafish models for functional validation of VUS in *COL1A2* and underscore their potential to elucidate pathogenic mechanisms. This approach could be adapted to study VUS in other genes, offering a valuable tool for genomic screening.

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P23.035.A Clinical Validation of a Haplotyped Whole Genome Sequencing Assay Using PacBio HiFi Sequencing

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Background: Next-generation sequencing (NGS) has enabled unprecedented insights into the molecular basis of health and disease. Short-read NGS have become integral to the diagnosis of genetic diseases, and in supporting clinical trials. Unfortunately, while current short-reads are cost-effective and scalable than ever before, they are limited in resolving complex genomic regions, detecting structural variations, and covering repetitive sequences. They do not accurately differentiate between critical *trans*/*cis* compound heterozygosity. While such challenges can be mitigated by long-read sequencing approaches, widespread clinical adoption is limited due challenges in sample handling, scalability, and cost.

Material and Methods: We report CLIA/CAP validation of a clinical long-read WGS assay for comprehensive genome-wide detection and phasing of SNVs, INDELs, CNVs, and CpG. High molecular weight DNA was extracted from blood, fragmented (~15–20 Kb), followed by SMRTbell library preparation, sequencing on PacBio Revio SMRTcell, to ~20–30x average coverage.

Results: Variant calling accuracy was assessed by comparing variant calls in healthy donors to those obtained by Illumina PCR-free WGS assay, as well as analysis of Genome In A Bottle (GIAB) well-characterized reference samples. The variant calling concordance F1 score was 0.99 and 0.98 for SNVs and INDELs, respectively. Phasing accuracy was performed by trio analysis on specific genes of interest.

Conclusion: This validated long-read WGS assay overcomes key limitations of short-reads, enabling comprehensive genome-wide detection and phasing of all variant classes. By leveraging automation and high-throughput workflows, this assay provides a powerful tool for precision genomic medicine, with the potential to improve diagnostic accuracy, treatment selection, and patient outcomes.

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P23.036.B An NGS salvage pathway for solid tumour testing

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Background: Genetic testing is a vital part of the diagnostic pathway for a growing number of cancers. High sensitivity and capacity to detect multiple variants simultaneously makes Next Generation Sequencing (NGS) panels the ideal testing solution. In Yorkshire and North-East Genomic Laboratory Hub (YNEGLH) Illumina's TruSight Oncology 500 High-Throughput (TSO500) NGS panel is used for solid tumour testing. However, for some panels over 25% of samples fail testing due to poor sample quality or limited material and the turnaround time of 3 weeks is too long if results are required urgently. Thermo Fisher's Oncomine™ Precision Assay (OPA) is a rapid, sensitive NGS pathway that requires less starting material.

Material and Methods:

- DNA and RNA TSO500 failed samples were tested on the OPA panel to assess sequencing quality and coverage.
- OPA detection of known DNA and RNA variants was assessed using control samples.
- Local turnaround times (TAT) for the TSO500 and OPA pathways were audited over a 3 month period.

Results:

- 76% low quality DNA or RNA samples passed OPA quality metrics.
- 100% DNA variants targeted by the OPA panel were detected.
- 100% RNA fusions were detected by the OPA targeted or imbalance fusion assay.
- OPA local TAT is at least 6 days shorter than the TSO500 TAT.

Conclusion: The OPA is a rapid assay which performs well with low quality samples, making it a viable salvage pathway for urgent or low-quality samples.

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P23.037.C Analysis of genetic edition efficiency rate on isogenic hiPSC subjected to Prime Editing CRISPR method

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Background: Human induced pluripotent stem cells (hiPSC) have great potential as experimental models on a variety range of fields, especially on those regarding gene edition. The aim of this study was to analyze the genetic edition efficiency rate of different CRISPR/Cas9 methodologies targeting genes associated with autism spectrum disorder (ASD) in hiPSC.

Material and Methods: hiPSC were cultivated in Essential 8 media, transfected using Lipofectamine Stem and submitted to single cell isolation using Hana cell dispenser. Prime Editing technique was employed to introduce 4 different ASD-associated missense mutations in 4 different genes (*POGZ, DEAF1, GRIN2A, SYNGAP1*). The dual-gRNA methodology was utilized to generate 30–60 kb gene deletions on the same genes. CRISPR oligos (pegRNA, gRNA) were designed using UCSC Genome Browser tracks and Prime Design tool. Genetic editing efficiency rate was calculated by dividing the number of edited colonies by the total number of colonies post-transfection.

Results: The Prime Editing efficiency rate was 0–22%, but most of the variants observed were indels in heterozygosis, and not a missense variant as planned. The amount of CRISPR oligos transfected impacted on the zygosity of the edited lines retrieved. The desired missense variants in heterozygosity were obtained with 0–2% editing rate. The dual-gRNA efficiency rate was 1–4%, all the editions obtained were aligned with the editing goals and in heterozygous state.

Conclusion: Prime Editing on hiPSC provides higher editing rates, although further optimization is required to retain patient-specific missense variants. Finally, the dual-gRNA editing method demonstrates presents with low editing efficiency but high specificity.

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P23.038.D Beta evaluation of a panel of *HBA1, HBA2* and *HBB*genes using ONT/AmplideX chemistry

Tony Yammine[1], *Paul A. Wadsworth*[2], *Nathalie Bonello-Palot*[3], *Chandler Ho*[2], *Serge Pissard*[4], *Ilona Volkova*[2], *Laura Feyereisen*[5], *Hélène Moret*[1], *Emilie Landais*[1], *Carlos J. Suarez*[2], *Anne-Sophie S. Lebre*[6]

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Background: For challenging genes involved in rare inherited diseases, non-NGS methods are still required, such as PCR/CE, qPCR, Gap PCR, MLPA or Sanger sequencing. To simplify user workflow, three genes involved in thalassemia have been pooled in a new Asuragen kit including *HBA1/2* and *HBB* genes (in Mix C). We evaluated the workflow on 57 known positive and 44 negative clinical samples collected from expert clinical laboratories and reviewed genotype agreement between the assay and orthogonal methods.

Material and Methods: We evaluated the Asuragen AmplideX Nanopore Carrier Plus Kit, combining the PCR enrichment technology with Nanopore DNA sequencing. Target genes were amplified by PCR (around 1 kb amplicons) from clinical samples. Libraries were prepared, pooled, and loaded on R10.4.1 Minion or Flongle flowcells. Automated analysis and reporting was performed using released Asuragen AmplideX One Reporter software.

Results: Mix C (*HBA1/A2/HBB* genes) Minion and Flongle tests will be presented. Multiple variant types were assessed, including SNV, Indel, or SV. Variant allele phasing was determined. Variant zygosity, sequencing accuracy, and average error rate of ONT sequencing were evaluated in comparison with current clinical diagnostic standards. Overall, >95% of samples with sufficient read depth passed quality control and of those, >95% were concordant with orthogonal methods.

Conclusion: The analytical performance characteristics of the Asuragen/ONT assay, along with its ease of use and turn-around time make it a promising method for clinical use. The clinical research implementation of this assay might simplify testing conducted for both diagnostic and carrier screening purposes.

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P23.039.A Whole-Genome Sequencing is a viable replacement for chromosomal microarray and Fragile X PCR testing

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Background: Chromosomal microarray analysis (CMA) is the first-line test for broad pediatric indications (global developmental delay, multiple congenital abnormalities). Fragile X testing is also performed for many of these same individuals. Whole genome sequencing (WGS) can change the paradigm by coupling CMA detection capabilities with robust small variant detection, while adding the ability to rule out expanded Fragile X alleles.

Material and Methods: A set of 102 previously tested samples initially analyzed by clinical CMA at Quest Diagnostics using Affymetrix CytoScanHD were subsequently sequenced at Broad Clinical Laboratory (BCL) using clinical 30x PCR Free WGS sequenced on Illumina NovaSeqX plus. Secondary analysis was performed using Dragen v4.3.6.

Results: 100% of the reportable events were detected by WGS. When applying the clinical CMA size filtering to the list of calls from WGS, WGS had an average of 21.89 events compared to 5.25 events from CMA. Several post processing approaches reduced the number of events for review with WGS to within +/− 10% of the number of events observed in CMA while still retaining the reportable event. For Fragile X, WGS detected trinucleotide repeats ≤45 with 100% sensitivity, and known expanded alleles were flagged as not normal in the 19 samples with known Fragile X allele status (Normal, Intermediate, Premutation, and Full).

Conclusion: In this work we demonstrated the clinical equivalency of WGS compared to traditional clinical CMA. We also highlight the additional advantages of WGS beyond CMA equivalency, including the ability to rule out Fragile X syndrome.

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P23.041.C Evaluation of Long-Read Whole Genome Sequencing Technologies and Tools for SV Detection

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[1]*Center for Cardiovascular Genetics and Gene Diagnostics, Swiss Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland,* [2]*Next Generation Sequencing Platform, University of Bern, Bern, Switzerland,* [3]*Laboratory of Functional Genome Analysis, Gene Center, University of Munich (LMU), München, Germany,* [4]*Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland.*

Background/Objectives: Compared to short-read sequencing (SRS), long-read sequencing (LRS) technologies provide advantages for detection of structural variants (SVs) in whole genome sequencing (WGS). Many SV callers exist but their performance differs significantly. We have optimised the preparation steps for PacBio and ONT libraries to minimise short fragment bias and tested several tools for their ability to detect a known set of challenging SVs due to multiple events or breakpoints in non-unique sequence regions.

Methods: Four DNA samples with challenging SVs previously detected by SRS (PCR-free WGS, PE150, 60x) were sequenced on a PacBio Revio as well as on an ONT PromethION platforms (30x) after library preparation optimisation. Sequencing data was analysed with the respective standard pipelines. CPU and GPU accelerated pipeline versions by Sentieon Genomics and Nvidia Parabricks were tested as alternatives. Several SV callers where applied and results manually reviewed using Integrative Genomics Viewer.

Results: Library preparation protocols including DNA shearing and size selection reduced the short fragment bias. Despite the observed differences in performance of the tested analysis pipelines and SV callers on challenging variants, LRS enabled the detection of breakpoints missed by SRS, although manual reviewing was required for full characterisation.

Conclusion: LRS can be the solution for breakpoint identification in repetitive regions and for characterisation of complex SVs not exceeding their read length. Improved SV callers and optimised library preparation protocols are vital to take full advantage of LRS in genetic testing. As current LRS technologies improve, they enable an improved future for clinical sequencing.

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P23.042.D The Evaluation of Oxford Nanopore Ultra-Long sequencing approach

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Background: Ultra-Long reads approach can improve de novo assembly and phasing, provide better insight into DNA sequence of repetitive regions, copy number variants and otherwise complex regions of the genome, and allows the identification of large chromosomal rearrangements and structural variants (SV). The longest known reads with this approach are over 4 Mb long.

Material and Methods: We extracted 6M cells per sample using Monarch Kit (NEB) from Ashkenazim trio reference cell lines (Coriell Institute), including HG002 individual characterised by the GIAB consortium. We prepared Ultra-Long libraries and sequenced them using PromethION R10.4.1 flowcells (ONT) with Super-accurate basecalling. We evaluated general quality metrics, SV calling performance compared to GIAB T2T reference, and assembly metrics using Hifiasm with optional HERRO correction.

Results: On average, we obtained 52 Gb of filter-passing throughput per flowcell, with an average N50 value of 56 kb, and the longest observed reads reaching >700 kb in length. Preliminary results indicate a slight improvement in F1 score for SV calling compared to standard ONT libraries. Assembly analysis is still ongoing but the error correction seems to improve both N50 metrics and the number of contigs.

Conclusion: The read length with N50 >50 kb has no competition on the sequencing market. Sufficiently mature bioinformatics tools are available for de novo assembly from Ultra-Long reads. On the downside, Ultra-Long approach requires large DNA input quantity that is incompatible with many sample types. The per-flowcell yield is lower than for standard ONT libraries but the chemistry continues to improve in both throughput and accuracy.

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P23.044.B *RFC1* analysis by genome and targeted long-read sequencing (PacBio)

Katharina Lackner[1], *Rebekka Entstrasser*[1], ***Katharina Herzog, PhD***[1], *Klaus Oberhuber*[1], *Roman Praschberger*[1], *Beatrix Mühlegger*[1], *Martina Witsch-Baumgartner*[2], *Johannes Zschocke*[1]

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Background: Homozygous or compound-heterozygous pentanucleotide repeat expansions or truncating mutations in the *RFC1*

gene lead to cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). Pathogenic expansions range from 250 to more than 2000 repeats, making the identification of the exact repeat expansion challenging. The newly released PureTarget Repeat Panel (PacBio) may overcome these challenges. However, for the detection of truncating mutations on the second allele a second method is required.

Material and Methods: We analysed the *RFC1* repeat region with genome long-read sequencing (PacBio) as well as the PureTarget CRISPR-Cas9 Assay (PacBio) which is designed for targeted analysis of multiple repeat regions. Data was generated by multiplex HiFi long-read sequencing on a Revio system (PacBio) and analysed either with our in-house pipeline or with the Pure Target Repeat Expansion Pipeline.

Results: Genome long-read sequencing detected shorter repeat expansions but failed to detect larger expansions due to low coverage. In contrast, PureTarget identified large RFC1-repeat expansions although for expanded alleles coverage is reduced.

Conclusion: PureTarget enables multiplex analysis for selected repeat regions and is applicable for very large expansions due to high coverage of approximately 200–300x but it is limited to those selected regions. In contrast, genome long-read sequencing only achieves a coverage of approximately 30x, which severely limits the possibility of detecting very large repeat expansions. However, the use of genome long-read sequencing enables the identification of RFC1 truncating mutations on the second allele. Additionally, it is suitable for the identification of mutations in other genes including (shorter) repeat expansions.

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P23.045.C Rapid, directional tagmentation with MosaiX TM: a novel workflow utilizing TnX engineered transposase for fast and high performing DNA library preparation

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Tagmentation using Tn5 transposase can be a highly streamlined approach to sequencing library preparation, although a number of quality challenges remain. Tn5 exhibits strong insertion-site sequence bias, leading to more uneven genome coverage. Additionally, typical methods use two different transposomes to add Read 1 and Read 2 adapters, generating libraries where half of the molecules are unusable due to having the same adapter on both ends, reducing overall complexity. Consequently, researchers often choose more time-intensive methods like enzymatic fragmentation with adapter ligation.

Here we present MosiaX, a novel library preparation chemistry that employs directional tagmentation with an engineered transposase, TnX, developed through directed enzyme evolution for improved activity, reduced insertion bias, and enhanced inhibitor tolerance. In this approach, DNA is fragmented and Read 1 adapters added to the 5′ end via TnX followed by 3' ligation of Read 2 adapters, generating a higher proportion of productive library molecules. The streamlined workflow produces ready-to-sequence libraries in just 90 minutes.

Benchmarking against bead-linked Tn5 tagmentation and enzymatic fragmentation in human exome and genome applications, we observe reduced insertion bias and improved complexity relative to Tn5, and equivalent quality to fragmentase-based methods. MosiaX also enables a realistic one-day exome workflow when combined with Twist's rapid hybridization kit, requiring less than 8 hours from starting DNA to captured libraries on sequencer. Additionally, we demonstrate MosaiXTM chemistry's versatility for PCR-free workflows, UMI incorporation, and compatibility with non-Illumina sequencers. This method addresses key quality limitations of Tn5-based approaches, offering a rapid workflow without sacrificing data quality.

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P23.046.D PCR-free, single-analysis approach for the rapid detection of variants in *PKD1* with targeted adaptive sampling long-read sequencing

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Background: NGS is the first-tier test for SNVs analysis, but ambiguous mapping of short reads limits the study of repetitive or duplicated regions. LRS can access and accurately map highly homologous sequences[1]. *PKD1* is characterized by high GC-content and has six pseudogenes with high sequence homology. NGS analysis of *PKD1* requires multiple steps, resulting in a labour-intensive protocol. Some authors have recently applied long-range-PCR and LRS assays to study *PKD1*, with promising results[2,3].

Material and Methods: We selected 7 patients with a known monoallelic mutation in *PKD1*. LRS was performed with Oxford Nanopore Technology (ONT) adaptive sampling, a software-based enrichment method that doesn't require additional library preparation. Reads were aligned with Minimap2 to the T2T-CHM13 human genome assembly. Variant calling was performed with Sniffles, Spectre and DeepVariant. Margin was applied for haplotag. Variants were annotated and prioritized with VEP and Rabdomyzer[4].

Results: We obtained reads with a mean N50 of 11279 bp and mean read accuracy of 98.65%. Mean coverage among all 7 samples was 23X. We accurately detected known variants in *PKD1* in all 7 samples analysed, identifying 6 SNVs and one intragenic deletion.

Conclusion: These results confirm the applicability of LRS for the study of genes with pseudogenes through a single level of analysis without PCR amplification, suggesting this technology as a feasible diagnostic tool. The use of targeted approaches might reduce the impact of sequencing errors by achieving higher coverage with lower costs. Further studies on larger cohorts are required before implementing this strategy into the clinic.

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G. Olivucci: None. **E. Iovino:** None. **T. Pippucci:** None. **M. Gut:** None. **M. Müller:** None. **I. Gut:** None. **P. Magini:** None.

P23.047.A An undiagnosed case solved with the help of genome wide DNA methylation profiling

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Background: DEGCAGS syndrome is an autosomal recessive disorder characterized by global developmental delay, dysmorphic facial features and immunodeficiency due to biallelic *ZNF699* variants. A specific DNA methylation profile has been recently associated with this syndrome.This study aims to describe the immunological features of a patient with DEGCAGS syndrome, in whom diagnosis was suggested through DNA methylation profiling.

Material and Methods: CGH-array, whole exome sequencing, clinical exome and sanger sequencing were used to define the genetic diagnosis. DNA methylation was used to assess the pathogenic role of a heterozygous de novo VUS in ADNP gene identified through clinical exome.

Results: The pathogenic role of the VUS in ADNP gene was ruled out through the study of DNA methylation episignature which, instead, suggested a diagnosis of DEGCAGS syndrome. This diagnosis was later confirmed by identifying a homozygous missense c.153C>A variant in ZNF699 through Sanger sequencing. Immunological studies revealed novel findings: a significant reduction of CD4+CD45RA+ naïve T cells (12%) and CD19+ B cells (2%, 33.2 cells/μL), along with impaired T cell proliferation. The patient experienced severe lung infections, suggesting a TlowB-NK+ combined immunodeficiency (CID). Despite severe B-cell depletion, immunoglobulin levels and antibody responses remained normal.

Conclusion: This report provides the first in-depth characterization of immunological abnormalities in DEGCAGS syndrome, indicating that biallelic loss-of-function ZNF699 variants may cause syndromic CID. DNA methylation episignature proved to be a valuable diagnostic tool, highlighting its potential to improve diagnostic yield in unresolved cases.

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P23.048.B The Clinician-reported Genetic testing Utility InDEx for genomic newborn screening (C-GUIDE NBS): Development and content validity

Robin Z. Hayeems[1,2], *Salma Shickh*[1], *Stephanie Luca*[1], *Elise Poole*[1], *Katharine Fooks*[1], *Amanda Pichini*[3], *Joanna Ziff*[3], *Katrina Stone*[3], *Bowen Xiao*[1], *Wendy J. Ungar*[1,2], *Pranesh Chakraborty*[4], *David Bick*[3]

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Background: Genomic newborn screening (NBS) initiatives are emerging worldwide. Evidence of clinical utility, acceptability and performance are required to guide policy and funding decisions. Tailored to the parameters of NBS and informed by the Clinician-reported Genetic testing Utility InDEX (C-GUIDE), we aimed to develop a novel tool to quantify the clinical utility of genomic NBS, called C-*GUIDE NBS*.

Material and Methods: We identified relevant domains and modified wording of the original C-GUIDE items to create a preliminary draft of *C-GUIDE NBS*. Interviews were conducted with clinicians involved in genomic NBS to assess the relevance, comprehensibility, and comprehensiveness of the items. We quantified the number of items clinicians accepted, modified, or rejected and synthesized qualitative feedback to inform a revised version. Interviewees completed content validity questionnaires to rate the relevance and clarity of each item on a 4-point Likert scale. Scores between 0 and 1 were calculated. We finalized *C-GUIDE NBS* using a 3-step international Delphi consensus process.

Results: Interviewees ($n = 22$) indicated that eight of the preliminary 11-items were acceptable for inclusion as is, two required modifications, and one was rejected. On the revised 10-item tool, item relevance and clarity scores <0.8 prompted further item revision for the consensus process. With 26 expert panelists, item consensus and stability were achieved for a final 7-item tool.

Conclusion: The rigorously developed, expert-informed *C-GUIDE NBS* tool provides a novel measurement strategy for capturing the clinical utility of genomic NBS. Validity testing is underway, following which *C-GUIDE NBS* will be available for licensed use.

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P23.049.C Towards equity evaluations in genomics: A case study on variant effect predictors

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Background: Understanding genomic variant differences across populations is essential for equitable health. Genomic research faces diversity and equity challenges, with individuals of European genetic ancestry over-represented in databases (78% as of 2019). This bias limits the applicability of findings to individuals of non-European genetic ancestry. Computational genomic tools, including variable effect predictors (VEPs), can perpetuate these inequities, necessitating robust frameworks to evaluate their performance across populations.

Materials and Methods: We conducted a rapid literature review into (1) the statistical methods to (1) characterise diversity in genomic data and (2) evaluate equitable performance with respect to genetic ancestry, focusing on VEPs. Guided by this review, we developed an equity evaluation framework for genomic tools like VEPs.

Results: The literature review identified three critical needs: (1) disambiguating genetic ancestry from sociopolitical constructs such as race and ethnicity, (2) integrating technical advancements in ancestry stratification into evaluations, and (3) improving tools to evaluate VEP performance across genetic ancestry. We used VEPs as a case study for the application of our proposed equity evaluation framework while considering these needs and identified challenges of limited diversity in reference databases used in genomic equity evaluations.

Conclusion: Promoting equity in genomics requires systematic frameworks that identify biases in computational models. This work establishes a foundation for rigorously evaluating VEPs across diverse genetic ancestries, addressing current data availability limitations. By identifying critical gaps and providing a clear direction for future evaluations, it supports more inclusive genomics and enables the development of more equitable computational models in genomics.

C.O.I. Disclosure:

L. Braeuninger: None. **B. Lehmann:** None.

P23.050.D A promising single DNA-based assay to test for hemoglobinopathies, congenital adrenal hyperplasia, cystic fibrosis, spinal muscular atrophy and severe combined immunodeficiency for newborn screening

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Background: Newborn screening is a public health program focused on early-onset conditions for which timely intervention can prevent a severe clinical outcome. Newborn screening programs mostly use biochemical assays, but those often require confirmatory testing using an independent assay due to the high number of false-positive/negative results. DNA-based tests can serve as second-tier options, but single-region assays such as qPCR and Sanger sequencing are time-consuming

Material and Methods: We are developing a SALSA® digitalMLPA™ assay that may be used to follow-up current newborn screening tests. digitalMLPA is a multiplex PCR-based technique with around 1,000 probes for the detection of copy number variants (CNVs) and selected single nucleotide variants (SNVs), using Illumina platforms for amplicon detection and quantification.

Results: The assay contains probes targeting genes associated with hemoglobinopathies (CNVs and 6 frequent pathogenic SNVs for *HBA1/2*; CNVs and 14 frequent pathogenic SNVs for *HBB*), congenital adrenal hyperplasia (CNVs and 11 frequent pathogenic SNVs for *CYP21A2*), cystic fibrosis (CNVs and 33 frequent pathogenic SNVs for *CFTR*) and spinal muscular atrophy (CNVs of exon 7 of *SMN1*). In addition, this assay contains probes targeting T-cell receptor excision circles (TRECs) for the detection of severe combined immunodeficiency. Although population-dependent, the variant detection rate for these conditions is >70–80%. Preliminary data demonstrate that the digitalMLPA assay confidently calls both CNVs and targeted SNVs in cell line-derived positive samples.

Conclusion: Our digitalMLPA assay could serve as a valuable DNA-based tool in a newborn screening setting, allowing testing of five treatable early-onset conditions at once.

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P23.051.A Sequencing of repeat expansions in molecular diagnostics: comparing hybridization-based and CRISPR-Cas9-based approaches

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Background: Diagnostics for repeat diseases are typically performed with fragment-length analysis or Southern blot as separate assays for each indication, which do not provide detailed information on interruptions, methylation status or mosaics. The goal is to streamline the lab workflow for diagnostics of different repeat diseases, while simultaneously gaining more information on these regions and comparing two different enrichment methods to evaluate their efficacy in this context.

Material and Methods: Twenty repeat regions of 16 samples were enriched with a customized hybridization-based panel (Twist Bioscience) and the CRISPR-Cas9-method (PureTarget kit, PacBio). Long-read sequencing was performed on a Sequel IIe (PacBio), and data were analyzed using SeqPilot software (JSI) and Tandem Repeat Genotyping Tool (PacBio).

Results: Repeat lengths of 14 samples sequenced with the PureTarget kit were compared to previous diagnostic results, which revealed a high concordance. Methylation was detected in *FMR1* of three samples and seven samples showed mosaics (including *FXN*, *FMR1*, *DMPK* and *CNBP* genes). Only five of the samples which were hybridization-based enriched, showed the same results as previous methods. The others were either poorly covered or lacked one of the expected alleles.

Conclusion: Long-read sequencing with the amplification-free enrichment method using CRISPR-Cas9 can reproduce results from gold-standard methods and provides additional information that can be highly valuable for diagnostics of repeat diseases. While hybridization might be suitable for shorter repeats (e.g. in *ATXN1*), it has some key limitations in the detection of longer repeats and cannot give any information on methylation status.

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P23.052.B SBX sequencing as a flexible RNA-seq toolkit: Enabling high throughput single cell transcriptomic profiling at scale

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Background: Recent technological advances have enabled transcriptomic profiling of single cells at increasing scale. Here, we leverage a novel next generation sequencing technology called Sequencing By Expansion (SBX) to achieve unprecedented throughputs for analysis of commonly used 10x Genomics single cell RNA sequencing kits.

Material and Methods: Libraries targeting 5–10k peripheral blood mononuclear cells isolated from healthy donors were prepared for SBX sequencing using an optimized workflow. Sequencing data was pre-processed to mimic paired-end reads expected by traditional bioinformatics pipelines. Sequencing throughput was assessed using the number of usable reads attained, defined as reads with valid barcodes that confidently mapped to the reference transcriptome or probe set.

Results: For the 10x 3′ kit, we obtained 3.2B usable reads in one hour of SBX sequencing, which was 7.5X throughput compared to an Illumina NovaSeqX run. SBX insert lengths were substantially longer, with 2B reads greater than 600 bp. For the 10x Flex kit, we obtained 7B usable reads in one hour of SBX sequencing, which was 15X throughput compared to Illumina. For all kits, SBX and Illumina sequencing resulted in highly correlated expression levels and detected cell types.

Conclusion: SBX sequencing demonstrated massively high-throughput across all tested kits, recovering billions of usable reads, whilst maintaining single-cell metrics comparable to those generated with Illumina sequencing. SBX's workflow presents a

solution for the escalating need for greater sequencing throughput in single-cell profiling experiments, thus facilitating the resolution of cellular subpopulations and the discovery of new biological insights.

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P23.053.C Dual-fraction extraction: Efficient isolation of DNA and RNA from a single sample for genomic applications

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Background: Independent recovery of DNA and RNA enables comprehensive genomic analysis, supporting applications such as pathogen detection, sequencing, and gene expression profiling. However, performing separate extractions for both nucleic acids can require large amounts of sample. A more efficient solution is needed to maximize the use of precious samples by isolating both DNA and RNA from a single sample.

Material and Methods: Here, we validate the Mag-Bind® DNA/RNA Kit, a nucleic acid extraction kit capable of eluting DNA and RNA in separate fractions from a single sample of up to 10 mg tissue or 1×10^6 cells. DNA and RNA were extracted from 5 mg of murine liver, kidney, brain, and lung tissue, as well as from 1×10^6 AD293 cells.

Results: The resulting DNA and RNA were of high-quality when analyzed via spectrophotometry. Suitability of the DNA and RNA for applications downstream was analyzed via qPCR and RT-PCR, both of which demonstrated successful amplification without inhibition.

Conclusion: The Mag-Bind® DNA/RNA Kit presents scientists with an efficient solution for obtaining both DNA and RNA suitable for extensive genomic analysis and downstream applications.

C.O.I. Disclosure:

S. Schmidt: A. Employment (full or part-time); Significant; Omega Bio-tek. **M. Memo:** A. Employment (full or part-time); Significant; Omega Bio-tek.

P23.054.D Integrating LLMs and knowledge graphs for Improved Data Interpretation

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Background: Large language models (LLMs) have become essential in early drug development, aiding in the prediction of mechanisms of action, toxicity, and other properties. However, their lack of transparency and tendency for hallucinations pose challenges. By integrating annotated databases and retrieval-augmented generation (RAG), hallucinations can be minimized. To support researchers, The Hyve integrated an LLM into the Open Targets Platform (OTP) using BioChatter, an open-source framework for biomedical LLM applications.

Material and Methods: A knowledge graph (KG) was built based on OTP data to enable RAG and improve transparency. A use case was designed to assess the LLM's ability to infer relationships not explicitly present in OTP. The question, "Which GO terms are most associated with asthma?" was chosen since OTP lacks direct disease-GO_term relationships. The LLM, powered by GPT-4o, was used to query the KG.

Results: The LLM generated an accurate Cypher query and retrieved the most associated GO terms for asthma: protein binding (7546 associations), plasma membrane (4482 associations), and nucleus (4099 associations). These results confirm the LLM's ability to process KG data transparently while avoiding hallucinations.

Conclusion: Although the LLM successfully retrieved valid GO terms, more specific terms like positive regulation of transcription by RNA polymerase II would be more informative for research. The KG's structure and schema significantly impact LLM performance. However, BioChatter demonstrates great potential for drug development by enabling researchers to extract insights without requiring expertise in database querying.

C.O.I. Disclosure:

L. van den Biggelaar: A. Employment (full or part-time); Significant; The Hyve.

P23.055.A Implementation of a multi node Beacon network for genomic data exchange in Italy

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Background: Beacon is a data protocol developed by the Global Alliance for Genomics & Health (GA4GH) for the secure interrogation of sensitive data (1). It facilitates the discovery and retrieval of genetic, phenotypic, and clinical data for stakeholders in healthcare and research. This project aims to establish a multi-node Beacon network in Italy to demonstrate its applications and provide deployment guidelines for national interested parties. This effort aligns with international initiatives, such as the Genomic Data Infrastructure (GDI) project, which supports genomic data exchange across Europe.

Material and Methods: A multi-node Beacon network was implemented using the Beacon v2 Production Implementation (B2PI) and the Beacon v2 Reference Implementation (B2RI) tools API. The network is currently populated with synthetic genomic and phenotypic data, and stringent tests have been conducted to evaluate data querying, retrieval efficiency, and network stability.

Results: We focused on deploying a multi-node network and establishing a functional testing environment. Initial tests assessed the network's performance in handling genomic queries and its potential for larger-scale projects. Based on these results, we drafted standard operating protocols for deployment.

Conclusion: By setting up and testing a Beacon network in Italy, this project promotes the adoption of international standards for genomic data sharing and contributes to EU-funded initiatives for genomic data discovery and exchange.

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F. Genova: None. **S. Diprima:** None. **R. Pini:** None. **P. Uva:** None. **M. Chiara:** None. **M.J. Morelli:** None.

P23.056.B Resolving variant phase with long-read Nanopore sequencing: a case of *MUTYH*-associated polyposis diagnosed as an incidental finding

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Background: A 35-year-old woman sought genetic counseling due to family history of breast and ovarian cancer. Her mother was diagnosed with breast cancer at age 45, ovarian cancer at age 55, and passed away at 65. Predictive testing using a next-generation sequencing (NGS) tumor panel was performed, revealing a variant of unknown significance in the *MSH2* gene and two heterozygous pathogenic variants in the *MUTYH* gene as incidental findings. *MUTYH*-associated polyposis (MAP) is an autosomal recessive condition[1], necessitating determination of whether the variants are *in cis* or *trans*.

Material and Methods: Due to lack of access to the father for segregation analysis, long-range PCR based Nanopore sequencing was used to determine the phase of the *MUTYH* variants. Nanopore sequencing enables the analysis of long DNA fragments, facilitating the resolution of phase ambiguity in cases with potential biallelic variants[2].

Results: Nanopore sequencing confirmed that the two *MUTYH* pathogenic variants were in trans, establishing a diagnosis of MAP. This diagnosis enabled tailored surveillance recommendations, including regular colonoscopies to manage the increased risk of colorectal cancer.

Conclusion: This case demonstrates the utility of long-read Nanopore sequencing in resolving the phase of genetic variants, which is critical for diagnosing autosomal recessive conditions such as MAP. Establishing variant phase allowed us to provide precise risk assessment and propose effective surveillance strategies. Our findings underscore the value of advanced sequencing technologies in clinical genetics, particularly when conventional methods, such as segregation analysis, are not feasible.

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P23.057.C MosaiXTM DNA Library Prep Kit for Enhanced Genotypability and Coverage Uniformity in WGS and WES sequencing

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Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) are widely used short-read sequencing methods in clinical genetics. WES offers high coverage in the coding regions and lower costs, while WGS provides broader coverage, including non-coding regions that may harbor disease-causing variants. Both technologies ensure higher per-base read quality compared to long-read sequencing, making them the standard for detecting clinically relevant variants. The choice between WGS and WES depends on specific clinical needs. A key challenge in next-generation sequencing (NGS) is the low mappability regions (e.g., pseudogenes), due to insertion-site bias and limited complexity of Tn5-based library preparation protocols. Optimized protocols can enhance mappability and genotypability, improving coverage uniformity, reproducibility, and variant calling, while simplifying the workflow and reducing the number of steps and the risk of errors. In this study, we evaluated the MosaiXTM DNA Library Prep kit from seqWell, which relies on directional tagmentation and an engineered TnX transposase for increase library complexity and reduced insertion site bias. We generated WGS and WES libraries (NA12878), the latter captured with Twist Exome 2.0 plus Comprehensive Exome Spike-in. Our results show that the MosaiXTM DNA Library Prep kit protocol achieves high coverage uniformity, strong genotypability, and excellent reproducibility across regions with varying GC content, while requiring fewer steps and less time compared to other library prep protocols. Additionally, the MosaiXTM DNA Library Prep kit demonstrated high precision in SNV and INDEL calling for NA12878 (Genome in a Bottle), closely aligning with its gold-standard variant set.

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P23.058.D Microarray analysis on Illumina Infinium GSA-24v3.0 BeadChip with focus on polymorphisms covered by forensic Verogen ForenSeq Kinteligence Kit provides thousands of reliable SNPs genotypes from degraded DNA with low input

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Background: DNA samples of ideal quality are not always available for genetic analysis, making it necessary to analyse degraded samples for kinship analysis.

Material and Methods: Control saliva samples were collected into Oragene kits and DNA was isolated. Cell-free DNA samples from sweat were obtained by rinsing the glass beads touched by study participants with Qiagen elution buffer without lysing corneocytes. The Illumina Infinium GSA24-v3.0 BeadChip microarray was used to analyse both high-quality saliva-derived DNA and degraded cell-free DNA samples at limited concentrations.

Results: Samples from two unrelated individuals were tested at different dilutions, enabling the study of 10,145 out of 10,230 forensic SNPs involved in the Verogen ForenSeq Kintelligence Kit. Quality parameters, in particular SNP call rates (SCRs) and concordance at the called site, were compared for saliva-derived and degraded sweat-derived DNA. The SCRs for the degraded samples were similar to or lower than those for the reference saliva samples of the same DNA concentration (e.g. for a concentration of about 0.01 ng/µl DNA the obtained SCRs ranged from 28.44 to 68.55% for degraded samples vs. 65.28% for reference saliva sample). However, degraded DNA showed a higher concordance with the reference sample from the same individual (above 59%) than with samples from other individuals (below 42%) after proper filtering.

Conclusion: In this pilot study, we demonstrated that Illumina Infinium GSA24-v3.0 microarrays can genotype thousands of SNPs from highly degraded DNA samples at low concentrations and suggest approaches to optimise quality control and filtering of the results obtained.

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P. Novotný: None. **V. Stenzl:** None. **D. Pokorná:** None. **P. Šenkyřík:** None. **M. Korabecna:** None.

P23.059.A Exploring the use of buccal swabs as a non-invasive sampling method for third-generation genetic sequencing

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Background: Third-generation sequencing (TGS) technologies demand starting samples that meet specific requirements, most commonly a DNA concentration higher than 10 ng/µL and a 260/280 absorbance ratio between 1.8 and 2.0[1].

In this study, Copan buccal FLOQSwabs® were evaluated both immediately after collection and after 12 months of storage at room temperature (RT) to determine if they meet the DNA parameters for successful TGS analyses.

Material and Methods: Five donors self-collected one buccal FLOQSwabs®, which was stored at room temperature (RT) for 12 months (T12). On the day of DNA extraction, the same donors self-collected another buccal FLOQSwabs®, which was immediately processed (T0) alongside the T12 samples. All samples were processed using QIAmp® DNA Mini Kit on QIAcube®, following lysis with NAO® Basket. DNA concentration was determined using NanoDrop™1000 and Qubit™ dsDNA BR fluorimetric assays, while DNA purity was assessed through 260/280 absorbance using NanoDrop™1000.

Results: All buccal FLOQSwabs® met the DNA concentration and purity requirements identified for successful TGS analyses (*Table 1*).

The unpaired t-test comparing T0 and T12 samples showed no significant differences in DNA concentration measured by Qubit™ ($p = 0.6049$) and NanoDrop™ ($p = 0.5550$).

Conclusion: Results suggest that buccal FLOQSwabs® can be effectively used for TGS, whether processed immediately after collection or following prolonged storage at RT, offering a non-invasive alternative to blood sampling. Further tests are currently ongoing to assess the molecular weight of the extracted DNA, aiming to verify if it exceeds 10 kbp.

Table 1

	T0 Samples	T12 Samples
Average DNA Concentration using Qubit™ (ng/µl) ± σ	43,54 ± 26,52	36,72 ± 9,93
Average DNA Concentration using NanoDrop™1000 (ng/µl) ± σ	56,92 ± 25,20	49,14 ± 12,75
Average 260 nm/280 nm Absorbance ± σ	1,89 ± 0,03	1,89 ± 0,02

C.O.I. Disclosure:

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P24 Treatments for Genetic Disorders

P24.001.B The Role of Antisense Oligonucleotides in Molecular Approaches to Developing Therapies for Ultra-Rare Genetic Diseases

Luciano Mottola[1,2], *Lorenzo Vaccaro*[1,3], *Mirella Massa*[1,3], *Gennaro Gandolfo*[1,2], *Sandro Banfi*[1,4], *Nicola Brunetti*[1,3], *Alessandro De Falco*[1], *Antonella De Matteis*[1,5], *Diego di Bernardo*[1,6], *Gennaro Gambardella*[1,6], *Vincenzo Nigro*[1,4], *Annalaura Torella*[4], *Davide Cacchiarelli*[1,3,7]

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Background/Objectives: The development of a functional genomic approach is critical for the treatment of many ultra-rare genetic diseases, especially in the absence of targeted clinical therapies. Once the causative genes of these diseases are identified, antisense oligonucleotides (ASOs) can be used as a molecular approach to develop personalized therapeutic solutions. ASOs are highly versatile molecules capable of targeting specific RNA transcripts to modulate their expression, effectively slowing or halting the progression of rare genetic diseases.

Methods: In line with this goal, we have developed different specific signalling systems in which ASOs induce either exon skipping or transcript degradation, depending on the specific disease-related gene variant. The reporter system utilises a luciferase vector into which the specific genetic variant is cloned, enabling us to perform functional assays to evaluate the effectiveness of ASOs. This vector will be co-transfected with antisense oligonucleotides in cellular models to conduct an in vitro luciferase-based functional assay. Two distinct strategies will be employed: the first focuses on transcript degradation, targeting variants that produce non-functional or dominant-negative mutant proteins. The second strategy involves exon skipping, designed for variants located in non-essential gene regions or in exons within in-frame splicing tracts.

Results: Every system will be analysed through luciferase assays and full-length RNAseq to determine the precise efficiency of each probe for future clinical purposes.

Conclusion: This strategy, if successful, will pave the way for the development of specific therapies for hundreds of genetic conditions for which efficient drug development is not possible to date.

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P24.002.C Functionalized bioactive nano-ink for precision siRNA therapy in *FGFR2*-related syndromic craniosynostoses

Federica Tiberio[1,2], *Salvati Martina*[1], *Noah Giacon*[1], *Alessia Vita*[1], *Luca Massimi*[1,2], *Gianpiero Tamburrini*[2,1], *Lorena Di Pietro*[1], *Ornella Parolini*[1,2], *Alessandro Arcovito*[1], ***Wanda Lattanzi***[1,2]

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Background: Heterozygous activating mutations of *FGFR2* gene result in different syndromes primarily characterized by craniosynostosis. We developed a moldable nanoparticle (NP)-laden hydrogel for in situ delivery of siRNA targeting *FGFR2* to restore the maladaptive osteogenic commitment of cells in skull sutures of syndromic patients.

Material and Methods: Calvaria-derived mesenchymal stromal cells were isolated as surgical waste from 6 non-related Crouzon patients. siRNA able to silence *FGFR2* mRNA were designed and

tested in patient cells. Functionalized PLGA-PEG-Bis-sulfone NPs were produced and characterized via dynamic light scattering and scanning electron microscopy. Their biocompatibility was assessed through cell viability and proliferation assays. The intracellular trafficking of NPs was examined through live imaging and confocal microscopy. The knockdown efficacy of siRNA-NP complexes was studied in vitro using qPCR and western blotting. Finally, PLGA-PEG-Bis-sulfone NPs were 3D-printed within a hydrogel matrix and tested in patients' cells.

Results: siRNA treatment modulated the FGFR2 cascade in patient cells, as demonstrated by reduced phosphorylation of FGFR2 (48%) and of its key effector ERK1/2 (77%). Upon treatment, RUNX2 protein levels decreased by 34%, with overall reduction of osteogenic markers' expression and bone matrix deposition. NPs widely distributed in cell cytoplasm without affecting viability and proliferation. Live-cell microscopy demonstrated sustained and prolonged intracellular release of PLGA NPs from hydrogel in cell culture. Once inside the cells, NPs released siRNA able to target and modulate the overactivation of *FGFR2*.

Conclusion: The proposed bioactive nano-ink could be a promising tool for controlled release of therapeutic RNA-based drugs.

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P24.003.D Retrospective analysis of clinical and radiological response to selumetinib in Italian children affected by neurofibromatosis type 1 and inoperable, symptomatic, plexiform neurofibromas

Claudia Santoro[1], *Maria Cristina Diana*[2], *Mariachiara Servedio*[3], *Elena Arkhangelskaya*[4], *Gianluca Piccolo*[5], *Andrea Santangelo*[6], *Angela Mastronuzzi*[7], *Antonella Cacchione*[8], *Maya El Hachem*[9], *Carmela Russo*[10], *Mario Cirillo*[11], *Irene Russo*[1], *Maria Colonnese*[1], *Ilaria Cecconi*[12], *Antonio Grasso*[13], *Maria Teresa Loiotine*[3], *Nicola Santoro*[3], *Mariachiara Resta*[14], *Carmela De Meco*[15], *Consolata Soddu*[16], *Eugenia Spreafico*[17], *Bartolomeo Rossi*[18], *Chiara Fossati*[19], *Silverio Perrotta*[1], *Teresa Perillo*[3]

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Background: Selumetinib,indicated in children aged ≥ 3 years with neurofibromatosis type 1**(**NF1) and symptomatic, inoperable, plexiform neurofibromas (PNs),reduces the volume of PNs,and improves quality of life(QoL) and pain.

Materials and methods:This retrospective, observational,independent,multicenter study on children with NF1(3–18 years) treated with selumetinib aimed to assess the clinical benefit rate (CBR) defined as the percentage of patients with complete response, or minor/partial response(MR/PR), progressive disease(PD) or stable disease(SD) according to REiNS and RANO criteria.We further evaluated safety, impact on pain,and performance status(ECOG).

Results: Seventy patients(39 males) were enrolled(median age 7.9 yrs).Target PNs were mostly in the head/neck(32%) and limbs(43%).The median PNs volume was 126.8 ml(5–3820 ml). They mostly caused pain(51%), deformity(18.5%), and motor dysfunction(16%) and received a median of 49 cycles(range 7.5–79).The radiological response is summarized in table.The median time to MR or PR was at 12 cycles(range 6–24),with the best response(−96%) at 24 cycles.These statistically significant associations emerged:PD at 12 cycles was positively associated with final PD and negatively with final CBR;SD and PR/MR at 6 cycles associated both with the SD and PR/MR at 12 months but also with the latter at the end of follow up;CBR at 12 months negatively associated with final PD and positively with final CBR. ECOG and pain significantly improved during therapy.Adverse events were mainly mild (78% ≤ grade 2)yet frequent(80%).

Conclusion: We report a strong CBR in NF1-children with PNs treated with selumetinib. Responses seen at 6 and/or 12 cycles may forecast the final response.

C.O.I. Disclosure:

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P24.005.B Contribution of 3D Instrumented Gait Analysis to the evaluation and follow-up of Mucopolysaccharidosis IVA (MPS IVA) patients under elosulfase alfa

Camille Porteret[1], *Steeve Brousseau*[2], *Claire Delleci*[3], *Elodie Blanchard*[4], *Emilie Doat*[5], *Didier Griffiths*[1], *Etienne Guillaud*[5], *Mathilde Majean*[2], *Patricia Reant*[6], *Christophe Richez*[2], *Guilhem Sole*[7], *Nadia Mehsen-Cetre*[2], *Cyril Goizet*[1], *Julien Van Gils*[1]

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Background: Mucopolysaccharidosis IVA (MPS-IVA) is a rare lysosomal storage disorder caused by biallelic pathogenic variants in *GALNS*. The natural history of attenuated MPS-IVA phenotypes remains largely unexplored, with limited data on long-term outcomes under enzyme replacement therapy (ERT). This study aimed to assess the clinical evolution of attenuated MPS-IVA patients over several years of follow-up.

Material and Methods: Four attenuated MPS-IVA patients were followed for 3 to 6 years in an expert center. We applied the Morquio A Clinical Assessment Program, incorporating three-dimensional instrumented gait analysis (3D-IGA), osteodensitometry, and heart MRI to evaluate disease progression and response to ERT with elosulfase alfa.

Results: All patients showed stabilization or improvement in motor function and gait parameters. 3D-IGA provided insights into postural patterns both at rest and during walking, highlighting compensation mechanisms related to pain, particularly in the lower limbs. Significant pain reduction was reported in patients with higher baseline levels. One patient, after an 8-month ERT interruption, experienced a decline in motor function but returned to previous performance levels two years after treatment reintroduction, underscoring the importance of uninterrupted therapy to preserve motor function.

Conclusion: Follow-up data from all four treated patients demonstrated at least stabilization of autonomy and gait patterns after years of treatment. These results suggest that 3D-IGA is useful to monitor motor dynamic function and clinical improvement under ERT in real-world conditions. The data could inform new individualized recommendations to improve patient adherence to long-term treatment, adapting follow-up strategies to the specific needs of each MPS-IVA patient.

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P24.006.C Preclinical studies on translational read-through-inducing drugs (TRIDs) as novel therapeutic options for patients with Fanconi anemia harboring premature termination codons (PTCs)

Roberto Valli[1], *Anca M. Hristodor*[2], *Enrico Cappelli*[3], *Elena Baldisseri*[2], *Giuseppe Montalbano*[1], *Giovanni Micheloni*[1], *Giovanni Porta*[1], *Annalisa Frattini*[4,5], *Silvia Ravera*[6], *Francesca Fioredda*[3], *Giuseppe Lippi*[7], *Carlo Dufour*[3], *Marco Cipolli*[2], *Valentino Bezzerri*[8,9]

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Background: Fanconi anemia (FA) is caused by mutations affecting DNA repair-associated FANC genes, with 20% of patients harboring nonsense mutations. Ataluren (PTC124) is a clinically approved translational read-through-inducing drug (TRID) for premature stop codons (PTC) with a well-established safety profile even in pediatric patients. Amlexanox is an anti-inflammatory drug able to stabilize mRNAs and induce read-through of PTCs.

Material and Methods: We compared the effect of ataluren and amlexanox in rescuing FANCA and FANCF protein synthesis in lymphoblastoid cell lines and fibroblasts from FA patients harboring nonsense mutations. We evaluated cell viability, chromosomal rearrangements after exposure to genotoxic and clastogenic agents (DEB test) and efficacy in reducing p53 levels.*FANCA* and *FANCC* mutated fibroblasts were used for micronuclei formation and lamin B1 expression studies.

Results: Ataluren restored FANCA and FANCF protein levels and p53 results significantly downregulated with associated improved cell viability and reduced chromosomal aberrations observed after DEB test. Amlexanox was partially effective only on FANCA restoration, while no effects were observed after DEB test. Moreover, ataluren significantly limited the generation of micronuclei in nonsense-mutated primary *FANCC* fibroblasts, restoring the expression of lamin B1.

Conclusion: This study paves the way for clinical development of TRIDs for FA treatment, indicating ataluren treatment as candidate approach to address the genetic instability and reduce the risk of malignant transformation in FA cells.

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P24.007.D Organoids as scalable models for splice modulation therapy development in Alport syndrome

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Background: Disease-causing non-coding variants are now regularly identified. Most of such variants affect mRNA splicing, eventually treatable using antisense oligonucleotides (ASOs). The development of reliable models for assessing variant pathogenicity and developing therapeutic options represents a fundamental need. Organoids are emerging as powerful tools for disease modeling. We identified several non-coding disease-causing variations in *COL4A5*, which encodes the α5 subunit of type IV collagen [α5(IV)], leading to X-linked Alport syndrome (XLAS), a hematuric nephropathy with glomerular basement membrane (GBM) abnormalities. In this study, we present a framework that combines patient-derived fibroblasts and kidney organoids to demonstrate the therapeutic efficacy of ASOs in XLAS patients.

Materials and methods: We generated an XLAS organoid model by introducing a severe deep-intronic variant in hiPSCs using CRISPR/Cas9 technology. To characterize the XLAS organoids, we employed a multi-omics approach alongside immunostaining. To quantify collagen α5(IV) deposition in the GBM, we developed a semi-automated Fiji macro for analyzing GBM collagen α5(IV) deposition.

Results: Using multiomics analysis and protein staining, we describe the development of GBM in wild-type and mutated human kidney organoids. We show that GBM maturation is a dynamic process that requires long organoid culture (culture up to day 38 of differentiation). Then, using automated quantification of

α5(IV) at basement membranes in organoids carrying the splicing variants identified in patients, we demonstrate the efficacy of ASO treatment for α5(IV) restoration.

Conclusion: These data contribute to our understanding of the development of GBM and pave the way for a therapeutic screening platform for patients.

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P24.008.A The PARADIGM project: advancing from a definitive diagnosis to a therapeutic option for ED and NMD patients

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Background: Reaching a definitive molecular diagnosis in patients with rare genetic diseases (RGDs) is crucial for proper management, counseling, and can be a starting point for the development of a therapeutic option for patients. RNA therapeutics have recently emerged as a promising tool for treating RGDs due to their ability to target almost any structure within the cell. Within the PNNR-PARADIGM project, focused on addressing missing heritability and developing therapies for RGD patients with eye disorders (EDs) and neuromuscular diseases (NMDs), we investigated three undiagnosed families to identify genetic causes and develop therapeutic approaches.

Material and Methods: genome sequencing, RT-PCR analyses, and mini-gene assays were used to identify and characterize four cryptic variants potentially affecting splicing in known ED genes. To target the splicing defects, we rationally designed 16 splice-switching antisense oligonucleotides (ssASOs) with a phosphorothioate backbone and 2'-O-methyl modifications. The molecules have been tested on patient-derived fibroblasts or HeLa cells transfected with mutant minigene by lipid-based transfection.

Results: The pathogenicity of a silent *ABCA4* variant, causing 35 bp intron retention was assessed by mini-gene assay, whereas RT-PCR analyses characterised three deep intronic variants in *CEP290* and *SDCCAG8* genes, resulting in pseudoexons formation. For each variant we identified at least one ASO able to restore normal splicing in vitro. Further testing of these molecules on disease-relevant 2D/3D models is ongoing.

Conclusion: Genetic diagnoses guide prognosis, counseling, and therapies. Our project provides proof of concept for effectively targeting disease-causing cryptic variants within the context of precision medicine.

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P24.009.B Tyrosine kinase inhibitors in Kosaki/Penttinen syndromes: New reports, follow-up of treated individuals and literature review

Céline Jost[1], *Knowing and Treating KOGS/PS*[2], *Alessandro Mussa*[3], *Jean-Emmanuel Kurtz*[4], *Ashmika Gokhul*[5], *Silvia Kalantari*[6], *Diana Carli*[7], *Andrea Gazzin*[6], *Thirona Naicker*[6], *Antoninette Chateaux*[5], *Yordi-Michaël Bouhatous*[1], *Liubinka Mirakovska*[1], *Théo Gaumet*[1], *Helena Iznardo Ruiz*[8], *Eulalia Baselga*[9], *José Manuel Mascaro*[8], *Cecilie Bredrup*[10], *Titas Gladkauskas*[11], *Cecilie Fremstad Rustad*[12], *Ingrid Voktor Svinvik*[12], *Dinel Pond*[13], *Elise Schaefer*[14], *Maxime Luu*[15], *Marc Bardou*[15], *Laurence Faivre*[16]

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Introduction: Heterozygous activator variants of platelet-derived growth factor beta (PDGFRβ) are associated two devastating heterogeneous ultra-rare conditions Kosaki overgrowth syndrome (KOGS), Penttinen syndrome (PS). PDGFR-targeted Tyrosine kinase inhibitors (TKIs), commonly used for hematological malignancies, are now considered potential treatments for KOGS/PS. Although five case reports have shown promising efficacy and safety, current data remain insufficient to fully assess the benefit/risk balance. The international consortium 'Knowing and Treating KOGS/PS' was established to evaluate real-life outcomes for treated and untreated individuals.

Materials and Methods: The consortium present three new cases of KOGS/PS treated with TKIs and update four previously published cases, from 6 countries.

Results: Individuals received between 6 months and 8 years of treatment (Imatinib N = 8/8, Dasatinib N = 3/8, Sunitinib N = 1/8) with an overall mean treatment duration of 43.86 months. Of the eight individuals treated, ages ranged from 6 to 57 years at the start of treatment, with five individuals starting treatment during childhood and three in adulthood. Most individuals (7/8) experienced improvement within months of starting treatment, with minimal side effects. However, some individuals encountered a plateau effect or a decrease in efficacy, prompting a switch to another TKI, possibly after functional tests.

Discussion: These early findings highlight the potential of TKIs in managing KOGS/PS. To enhance monitoring and data collection, standardized follow-up protocols and an electronic case report form have been introduced. This initiative aims to

systematically compare the natural history of treated and untreated individuals, compensating for the rarity of the disease, which makes conducting formal clinical trials challenging.

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P24.010.C Prime editing-mediated correction of the *OPTN* gene in ALS patient-derived fibroblasts

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by motor neuron degeneration. ALS Type-12 is associated with biallelic mutations in the OPTN gene, leading to neuronal dysfunction. Prime Editing (PE), a CRISPR-based gene editing technology, enables precise correction of point mutations without inducing double-strand breaks or requiring a donor template. This study aims to correct the c.1242+1G>A mutation in the OPTN gene in patient-derived fibroblast cells using PE and to evaluate its efficiency in vitro.

Materials and Methods: A patient diagnosed with ALS Type 12 underwent Next-Generation Sequencing (NGS), which identified the c.1242+1G>A variant in the OPTN gene. A prime editing guide RNA (epegRNA) was designed and cloned for precise base correction. Subsequently, the epegRNA-containing plasmid was transformed into chemically competent E. coli for plasmid amplification. Patient-derived fibroblast cells were cultured and transfected using Lipofectamine 3000 with comprising SpCas9 nickase, reverse transcriptase, and an epegRNA targeting the mutation. Gene editing efficiency was assessed via high-resolution melting (HRM) curve analysis.

Results: Post-transfection, HRM curve analysis confirmed precise nucleotide correction, with an estimated 30% editing efficiency at the target site.

Conclusion: This study demonstrates the feasibility of using Prime Editing to correct the *OPTN* c.1242+1G>A mutation associated with ALS Type 12. While Prime Editing has primarily been explored in a limited number of diseases, these findings suggest its potential as a therapeutic strategy for rare genetic disorders. Further preclinical and functional studies are required to validate its long-term efficacy and assess its potential for clinical translation

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P24.011.D Impaired Differentiation Capacity of MSCs in Mandibular Hypoplasia, Deafness, Progeroid Features, and Lipodystrophy (MDPL) Syndrome: An In Vitro Study

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Background: MDPL syndrome (OMIM #615381) is a rare autosomal dominant disorder caused by an in-frame deletion in the *POLD1* gene, encoding the catalytic subunit of DNA polymerase delta. It is characterized by abnormal white adipose tissue (WAT) distribution and mandibular hypoplasia, likely linked to bone-related issues. Previously, we identified early anomalous senescence in mesenchymal stem cells (MSCs) derived from MDPL-hiPSCs. This study investigates the differentiation potential of MDPL-MSCs into adipocytes and osteocytes, as a target of disease.

Material and Methods: For adipogenesis, MSCs were cultured for 21 days in MEM-alpha with specific supplements, including IBMX, dexamethasone, insulin, indomethacin, and rosiglitazone. For osteogenesis, MSCs were cultured in DMEM supplemented with FBS, dexamethasone, ascorbic acid 2-phosphate, and β-glycerophosphate. Gene expression was analyzed using RT-qPCR and TEM was used for ultrastructural analysis

Results: MDPL-MSCs exhibited a fivefold reduction in lipid droplet (LD) accumulation and smaller LDs compared to controls. Genes involved in lipid and glucose metabolism (*PPARγ, ADIPOQ*, *GLUT4*, and *FABP4*) showed decreased expression levels at transcript levels. Osteogenic differentiation demonstrated a decrease in calcium deposition in patients compared to age-matched controls as well as *ALP*, *RUNX2* and *OSTEOCALCIN* gene expression. TEM analysis revealed fewer electron-dense vesicles, nuclear deterioration, and autophagy-like macrovesicle aggregates, confirming cellular senescence.

Conclusion: MDPL highlights critical links between senescence and tissue differentiation. Dysregulation of the adipo-osteogenic balance has been associated with several pathophysiological processes, including aging, obesity, and osteoporosis. The observed defects provide a valuable model for understanding fundamental biological processes and identifying specific therapeutic targets for clinical applications.

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P24.012.A A UK platform for nucleic acid therapy for rare diseases - the UPNAT consortium

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Introduction: Nucleic acid therapeutics (NATs) offer great potential to treat rare diseases by targeting specific genetic causes. The rise in NAT clinical trials and approved therapies highlights their role in research to transform new ideas into new treatments and unique opportunities for preclinical and clinical studies, in which the UK has a high level of expertise.

Methods: UPNAT is the UK Platform for Nucleic Acid Therapy for rare diseases, funded by the MRC and NIHR as part of the Rare Disease Research UK Platform. UPNAT aims to advance NAT development by

creating essential resources and a national network, establishing a UK framework for NAT delivery. Key collaborators include NIHR BRCs, the UKRI NATA Hub and Genomics England.

Results: Working groups for NAT development:

1. **Target Selection**: This group is formulating guidelines for selecting genetic variants, target genes, applicable disease conditions and suitable patients for NAT and piloting their implementation within NHS services. A draft guideline has been developed and UPNAT has contributed to finalising the recently published N1C variant guidelines.
2. **Preclinical Development on individualized ASO therapy for ultra-rare diseases**: Collaborating with academic groups and international NAT consortia, the team is exploring effective pathways for developing individualised NATs at scale.
3. **Clinical Translation**: The team is streamlining clinical translation and regulatory approval by establishing data-sharing initiatives, offering training opportunities, and facilitating the creation of a NAT adverse event registry.

Conclusion: UPNAT seeks to accelerate NAT development for rare disease patients in the UK and contribute to international efforts advancing NATs.

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P24.013.B Preclinical Applications of Lentiviral Gene Therapy in X-linked Agammaglobulinemia (XLA)

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Background: XLA is associated with mutations in Bruton tyrosine kinase (BTK) and is an ideal candidate for hematopoietic stem cell(HSC)-based gene therapy. Previously, we demonstrated in vivo correction of immune defects in BTK-knockout mice using lentiviral vectors carrying coBTK. Here, we aimed to correct the B-cell deficiency in NOD/SCID/IL2Rγ-/- (NSG) mice, by self-inactivating lentiviral vectors.

Material and Methods: CD34+ cells were isolated from bone marrow (BM) samples of three patients (P1, P2, P3) and transformed in vitro with coBTK lentiviral vector. Transduced and untransduced cells were transplanted into a total of 20 NSG mice. The development of CD19+cells in bone marrow (BM), peripheral blood (PB), and spleen were evaluated by flow cytometry and qPCR by the end of 5th week following transplantation.

Results: CD19+ engraftment was detected by flow cytometry. In bone marrow and spleen, mice transplanted with transduced stem cells had a higher number of CD19+ cells compared to non-transduced stem cells. coBTK mRNA expression was evaluated by qPCR, and detected in all samples. The mice transplanted with the transduced cells of P1 showed the most significant mRNA expression in blood and spleen($p < 0.001$).

Conclusion: The patients' stem cells were transduced, and the expression of human cells started in the relevant tissues but could not yet be detected at the protein level by flow cytometry. Our findings suggest that the approach is efficient, but the engraftment process is not extended enough to translate the new genes into functional proteins detectable by flow cytometry.

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P24.014.C Innovative therapies for *FGFR3*-related skeletal dysplasias: treatment with Vosoritide in children with Achondroplasia. The experience at Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milan, Italy

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Background: Achondroplasia is the most common skeletal dysplasia, characterized by disproportionate short stature. It affects over 360,000 people worldwide and may lead to medical complications, functional limitations, and psychosocial challenges. It is caused by a gain-of-function mutation in the *FGFR3* gene, resulting in impaired endochondral ossification. Until now, the only effective treatment for achondroplasia has been limb lengthening. Vosoritide is the first pharmacological treatment approved for achondroplasia. It is an analog peptide CNP that counteracts the effects of the *FGFR3* mutation, promoting endochondral bone growth. Clinical trials have shown that vosoritide improves annual growth velocity in children with achondroplasia.

Material and Methods: We prospectively collected data from periodic evaluations of 27 children treated with vosoritide, focusing on changes in key anthropometric parameters relevant for auxological monitoring, as well as data on potential treatment-related adverse events or variations in biochemical parameters used in screening.

Results: Our data demonstrated an increase in height, confirmed by an improvement in the height Z-score compared to reference growth curves. Most notably, There was an increase in annual growth velocity at 18 months post-treatment compared to baseline by approximately 2.5 cm. This is consistent with findings from recent studies on similar cohorts, particularly in individuals with achondroplasia who had poor baseline growth velocities, averaging below 5 cm per year. The most common adverse events during the study were injection-site reactions and injection-site erythema.

Conclusion: Our data confirmed findings observed in other similar cohorts, with no significant sex-related differences. However, emerging data reinforce its clinical utility.

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P24.015.D Mass spectrometric profiling reveals alterations in N-Glycans in Tay-Sachs disease under autophagy-induced conditions

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Background: Tay-Sachs disease is a rare neurodegenerative disorder caused by mutations in the *HEXA* gene. *HEXA* gene encodes for the α-subunit of enzyme β-hexosaminidase A, which degrades GM2 ganglioside. Our research group developed a novel Tay-Sachs disease mouse model (*Hexa-/-Neu3-/-*) exhibiting neuropathological and clinical abnormalities. Previously, we identified impaired autophagic flux in the brains of *Hexa-/-Neu3-/-* mice. Moreover, we demonstrated autophagosome clearance in Tay-Sachs disease cells under lithium-induced conditions. Here, we aimed to evaluate changes in N-glycans in Tay-Sachs disease cells under autophagy induction and examined whether changes in glycan influence ER stress.

Material and Methods: The N-glycans profile was analyzed using LC-MS/MS in fibroblasts and neuroglial cells of 5-month-old *Hexa-/-Neu3-/-* mice and neuroglial cells of Tay-Sachs patients under lithium induction and nutrient deprivation. Additionally, the expression levels of ER stress-related markers were assessed in these cells using qRT-PCR and Western Blot analyses.

Results: We discovered a significant accumulation of high-mannose N-glycans, including Hex10HexNAc1, Hex8HexNAc1, and Hex9HexNAc1, as well as elevated expression levels of ER stress-related markers, CHOP and ATF-6, in Tay-Sachs disease cells compared to control groups. We found that autophagy induction causes degradation of accumulated high-mannose N-glycans associated with the downregulation of ER stress-related genes in Tay-Sachs disease cells.

Conclusion: Our study is the first to show altered high-mannose N-glycans in Tay-Sachs disease cells and suggests the presence of ER stress-mediated autophagy. Therefore, targeting N-glycans through autophagy induction may provide potential treatment strategies for Tay-Sachs disease in future studies.

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P24.016.A Investigation of the therapeutic potential of intrathecal human Neu3 sialidase delivery in Tay-Sachs disease mouse model

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Background: Tay-Sachs disease is a severe neurodegenerative disorder caused by mutations in the *HEXA* gene, which encodes the α-subunit of the β-hexosaminidase A (HexA) enzyme. A deficiency in HexA leads to the excessive accumulation of GM2, resulting in progressive neurodegeneration in individuals with Tay-Sachs disease. Our group developed *Hexa-/-Neu3-/-* mice, closely mimicking the neuropathological and behavioral changes with a shortened lifespan of 20 weeks. While murine sialidase Neu3 has been shown to promote GM2 degradation through a "bypass" mechanism in *Hexa-/-Neu3-/-* mice, the potential role of human Neu3 (hNeu3) in GM2 degradation remains unclear. Here, we aimed to explore human Neu3-based gene therapy using AAVrh10-hNeu3 in the *Hexa-/-Neu3-/-* mice pathology.

Material and Methods: Molecular biological and immunohistochemical analyses were performed to evaluate the therapeutic efficacy of single-dose intrathecal administration of AAVrh10-hNeu3 to 8-week-old *Hexa-/-Neu3-/-* mice.

Results: We observed an extended lifespan in *Hexa-/-Neu3-/-* mice, reaching up to 28 weeks, and improved weight and physical appearance. We also noted higher hNeu3 expression in the cortex and cerebellum of 20- and 28-week-old *Hexa-/-Neu3-/-* mice administered with 10^{11} vg/µl of AAVrh10-hNeu3. Immunohistochemical analyses revealed decreased GM2 accumulation and LAMP1 levels, alongside increased CNPase levels in 28-week-old *Hexa-/-Neu3-/-* mice compared to those of 20-week-olds across various brain regions.

Conclusion: Our findings suggest that upregulating human Neu3 expression in Tay-Sachs patients might be a promising therapeutic strategy for reducing GM2 accumulation and treating Tay-Sachs disease.

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P24.018.C Exploring RNA-based strategies to rescue haploinsufficiency in a form of neurodevelopmental disorders linked to *STX1B* gene

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Background: The *STX1B* gene (*OMIM#601485*) encodes Syntaxin 1B, a key protein in neurotransmitter release, via the SNARE complex. Syntaxin 1B drives the fusion of synaptic vesicles with neuronal membranes. Variants in *STX1B* causing haploinsufficiency are linked to a form of early-onset neurodevelopmental disorders (NDDs) characterized by GEFSP9+, myoclonic-atonic epilepsy and developmental delay. This study aims to explore the therapeutic potential of RNA-based technologies, including SINEUPs - a new class of synthetic antisense long non-coding RNA (lncRNA) - and CRISPRa, a gene-editing approach using a deactivated Cas9 protein fused to transcriptional activators. These approaches aim to increase STX1B protein levels and reduce the downstream effects of haploinsufficiency.

Material and Methods: qPCR and Western Blot to evaluate the expression of *STX1B* at mRNA and protein level in neuronal-like cellular models; in silico analysis of RNA structure of *STX1B* mRNA; design and synthesis of SINEUPs targeting the regulatory regions and the coding sequence of *STX1B*; application of VPR and SAM CRISPRa strategies targeting *STX1B* gene.

Results: CRISPRa approaches using VPR and SAM systems significantly upregulate the *STX1B* gene expression at transcriptional level. Furthermore, we are currently setting up experimental conditions to screen SINEUPs targeting the human *STX1B* mRNA.

Conclusion: RNA-based therapies offer a promising approach for addressing neurodevelopmental disorders caused by haploinsufficiency. Our preliminary data suggest that the CRISPRa tools

holds significant potential for upregulating gene expression. Combining transcriptional and post-transcriptional gene modulation to restore protein levels in *STX1B*-related synaptopathies may establish a foundation for future therapeutic developments.

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P24.019.D Absence of neurological manifestations in triple knock-out mice model of Tay-Sachs disease

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Background: Tay-Sachs disease is a lysosomal storage disorder resulting from mutations in the *HEXA* gene encoding the α subunit of lysosomal ß-hexosaminidase A enzyme that hydrolyzes GM2 to GM3 ganglioside. *Hexa-/-Neu3-/-* mice show the clinical manifestations of early-onset Tay-Sachs disease, including shortened lifespan, abnormal GM2 accumulation, neuroinflammation, neurodegeneration, and cognitive and motor function deficits. *B4Galnt1* gene encodes Beta-1,4-N-acetyl-galactosaminyltransferase-1 enzyme that facilitates the transfer of GalNAc to GM3 and GD3 to produce GM2 and GD2, respectively. In this study, we aimed to explore the neurological courses of *Hexa-/-Neu3-/-* mice by eliminating the *B4Galnt1* gene in triply-deficient mice.

Material and Methods: The cortex and cerebellum brain regions of 5-month-old *WT*, *Hexa-/-*, *B4Galnt1-/-*, *Hexa-/-Neu3-/-*, and *Hexa-/-Neu3-/-B4Galnt1-/-* mice were analyzed for neuronal death, astrogliosis and microgliosis using immunohistochemical stainings, ganglioside levels using thin-layer chromatography, and neuroinflammation-related gene expression levels using qRT-PCR.

Results: We found a significantly reduced neuronal death in *Hexa-/-Neu3-/-B4Galnt1-/-* mice compared to *Hexa-/-Neu3-/-* mice. We showed that excessive GM2 accumulation is absent in *Hexa-/-Neu3-/-B4Galnt1-/-* mice. We determined that elevated expression levels of neuroinflammation-related genes in *Hexa-/-Neu3-/-* mice were decreased in *Hexa-/-Neu3-/-B4Galnt1-/-* mice. Immunohistochemical stainings also revealed that activated astrogliosis and microgliosis in *Hexa-/-Neu3-/-* mice were notably reduced in *Hexa-/-Neu3-/-B4Galnt1-/-* mice.

Conclusion: Our study indicates that eliminating the *B4Galnt1* gene in *Hexa-/-Neu3-/-* mice rescues the neuroinflammatory and neurodegenerative symptoms of the disease. Our findings propose that the *B4Galnt1* gene might be a therapeutic target for treating Tay-Sachs patients in the future.

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S. Yanbul: None. **T.U. Çalışkan:** None. **M.C. Turali:** None. **V. Seyrantepe:** None.

P24.020.A Intracerebroventricular administration of yeast-produced human recombinant lysosomal β-Hexosaminidase partially corrects GM2 ganglioside accumulation in the Tay-Sachs mouse model

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Background: Tay-Sachs disease is a rare lysosomal storage disorder caused by mutations in the *HEXA* gene encoding α-subunit of β-Hexosaminidase A, leading to GM2 ganglioside accumulation in the central nervous system. Abnormal GM2 ganglioside accumulation causes neuropathology and leads to premature cell death in Tay-Sachs patients. Multiple therapeutical strategies have been investigated, including enzyme replacement therapy for TSD; however, there is still no effective cure (1). Previously, the efficacy of human recombinant lysosomal β-Hexosaminidases (rhHex-A) produced in yeast *P.pastoris* has been investigated in patient's fibroblasts (2). Here, we aim to explore the therapeutic potential of intracerebroventricularly (ICV) administered yeast-produced rhHex-A in a validated mouse model of Tay-Sachs disease (*Hexa-/-Neu3-/-*).

Material and Methods: The enzymatic activity of rhHex-A was measured in *Hexa-/-Neu3-/-* brain to determine its half-life. Thin-layer chromatography (TLC) and RT-PCR analysis and immunofluorescence (IF) staining with anti-GM2 / anti-LAMP1 were performed to show whether ICV-administered rhHex-A affects the GM2 accumulation and neuroinflammation in the cortex and cerebellum.

Results: TLC analysis and IF staining showed that rhHex-A administration reduced accumulated GM2 gangliosides in the cortex and cerebellum but did not extend the lifespan of the TSD mouse model. On the other hand, RT-PCR and IF staining demonstrated that rhHex-A prevents the elevated expression levels of neuroinflammation-related genes associated with astrogliosis and microgliosis.

Conclusion: Our results suggest that rhHex-A produced in yeast is functional in degrading accumulated GM2 ganglioside. However, multiple ICV administrations might be necessary to improve the neuropathology and extend the lifespan of the TSD mouse model.

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P24.022.C Offering expanded carrier screening (ECS) in the UK: do professional and policy stakeholders think it is worthwhile?

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Background: Expanded carrier screening (ECS) can identify, before conception, whether people with no known risk may pass on pathogenic variants to their children. Despite its increasing use in the UK, mostly in the private sector, there remains a lack of data and limited debate regarding the potential implementation of ECS. The aim of this study was to explore the views of key health professionals, policy experts and commercial providers on the current UK ECS offerings.

Material and Methods: Semi-structured interviews were conducted over 18 months (August 2023 - January 2025) with a purposive sample of key stakeholders with a remit or interest in providing or regulating reproductive genomic testing. Interviews were analysed with Thematic Analysis.

Results: Our study sample comprised 33 participants: 9 policy, 16 clinical and 7 commercial stakeholders. Two thirds of participants (24/33) believed ECS had significant benefits, including reducing transmission risks, increasing reproductive autonomy; and as a defence against legal liability in the IVF sector, saying it should be available for purchase if couples wanted it. Six

participants (6/33) were neutral or undecided, saying it was complex to assess the value of ECS with inconsistencies across panels and without a proper cost-benefit analysis. Only three viewed ECS as having limited utility while criticising it for increasing demand for NHS genetics services.

Conclusion: Most stakeholders were positive about ECS value, when offered with adequate information and support. However, they reported a seeming lack of concern about these issues at the national level.

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P24.025.B Comprehensive microRNA-seq transcriptomic analysis of Tay-Sachs disease mouse neuroglia revealed distinct miRNA profiles

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Background: Tay-Sachs disease is a rare lysosomal storage disorder that results in the progressive accumulation of GM2 predominantly in the central nervous system due to mutations in the *HEXA* gene encoding the α subunit of the ß-Hexosaminidase A enzyme. The mouse model of Tay -Sachs disease (*Hexa-/-Neu3-/-)* exhibited neurodegeneration and neuroinflammation along with the expression of pro-inflammatory cytokines and chemokines, severe astrogliosis, and microgliosis. Here, we aim to get more insight into the disease pathology by investigating whole miRNA profiles in *Hexa-/-Neu3-/-* neuroglia.

Material and Methods: Next-generation sequencing is used to assess the entire miRNA profile of *Hexa-/-Neu3-/-* neuroglia. 1966 mouse miRNAs were screened to detect differentially expressed miRNAs. These miRNAs and their targets were identified by comparative analysis of KEGG and REACTOME-enriched pathways.

Results: We identified for the first time that significantly upregulated miR-708-5p, miR-672-5p, miR-204-5p, miR-335-5p, and miR-296-3p; downregulated miR-10b-5p, miR-615-3p, miR-196a-5p, miR-214-5p, and miR-199a-5p among 174 differentially expressed miRNAs in *Hexa-/-Neu3-/-* neuroglia compared to age-matched *WT* neuroglia. Gene enrichment analysis revealed that several target genes are implicated in key pathways related to the neuronal system, neuroinflammation, and sphingolipid metabolism associated with the pathogenesis of Tay-Sachs disease.

Conclusion: We explored up- and down-regulated miRNAs that might be associated with the neuropathological manifestations of the disease in Hexa-/-Neu3-/- mice. Our results suggest that miRNA-based therapeutic approaches could be used to improve the clinical status of Tay-Sachs disease patients.

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P24.026.C A functional and molecular approach to address the heterogeneity of the response to CFTR modulators of the N1303K variant

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Background: Cystic fibrosis (CF) is a common genetic disorder caused by loss-of-function variants in the *CFTR* gene. CFTR modulators have been developed to correct CFTR defects and are approved for specific genotypes. The N1303K variant, the second most common CF-causing variant among Italian CF patients, exhibits trafficking and gating defects with variable response to modulators, allowing categorization into different groups based on drug response. Considering this variability, we aimed at investigating the molecular basis underlying these diverse responses to modulators of the N1303K variant.

Material and Methods: We performed a qualitative and quantitative evaluation (qPCR) of *CFTR* expression in N1303K-carrying epithelia differentiated from human nasal cells of CF and control individuals. A preliminary bulk RNA sequencing study has been also applied to selected samples.

Results: We found that *CFTR* mRNA levels are similar in all the controls and N1303K samples, except for a CF sample homozygous for the variant, which showed high basal activity further increased upon modulators treatment for which a deep molecular characterization is ongoing. Moreover, an integration of *locus*-specific expression data with the results of RNAseq analysis is also ongoing.

Conclusion: We have previously demonstrated that N1303K epithelia show different sensitivity to CFTR modulators and that this is due to intrinsic features of the epithelia. To address the molecular basis of such variability, we provided a quantitative and qualitative profile of *CFTR* expression in such samples. Our study may guide the development of novel pharmacological approaches for CF patients carrying the N1303K variant, currently lacking effective therapies.

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P24.027.D Potential benefit of biotin treatment in *HSPD1* related disorders

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Background: In 2004, a one-year-old boy presented with gastroenteritis, vomiting, lethargy and failure to thrive. Metabolic analysis of urine organic acids suggested holocarboxylase synthetase deficiency (HLCSD). Treatment with biotin commenced and symptoms improved, supporting working diagnosis of HLCSD. Patient later regressed and currently has severe intellectual disability, autism, epilepsy, and spastic paraplegia. Patient is fed through a g-tube and confined to wheelchair. Negative family history. Genetic re-evaluation was performed in 2023.

Material and Methods: Genomic trio-DNA was extracted from peripheral blood and sent to a reference laboratory for whole-exome sequencing.

Results: A de novo heterozygous likely pathogenic variant in *HSPD1* (NM_002156.5:c.1231G>C p.(Asp411His)) was detected. No pathogenic variants detected in *HLCS*.

Conclusion: The HLCSD working diagnosis was refuted. *HSPD1* is associated with autosomal recessive hypomyelinating leukodystrophy-4 (HLD4, OMIM #612233) and autosomal dominant spastic paraplegia-13 (OMIM #605280), with HLD4 being a better fit for the patient. The encoded protein of *HSPD1*, HSP60, forms a double-ring assembly of heptameric subunits. A dominant-negative effect could therefore explain the patients´ genotype-phenotype. Prior testing had shown heterozygosity for a *BTD* variant with 50% residual activity, and plasma biotinidase activity was 65% of normal median value. The possible contribution of the *BTD* variant on the patient's phenotype is unclear. According to published in vitro research, knockdown of HSP60 resulted in a 60% increase in reactive oxygen species levels which was ameliorated with biotin. This data supports the observed clinical improvement in the patient following biotin treatment. Further research on biotin therapy in *HSPD1* related disorders is warranted.

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P24.028.A Biomarkers for hereditary transthyretin amyloidosis: a longitudinal study in Bulgarian Glu89Gln carriers

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Background: Hereditary transthyretin amyloidosis (ATTRv) is an autosomal dominant genetic disorder caused by pathogenic missense variants in the *TTR* gene, leading to formation of transthyretin amyloid fibrils. Clinical phenotypes vary from progressive sensorimotor and autonomic neuropathy to infiltrative cardiomyopathy.There is a Bulgarian endemic region with a high *TTR* pathogenic variant frequency c.325G>C, p.Glu109Gln (Glu89Gln) (a rare founder pathogenic variant).Neurofilaments (NFs) are found in large quantities in axons in the central and peripheral nervous system.The ubiquity of NFs in axons explains their elevated release following axonal damage by neurodegenerative processes. However, the accuracy of NfL has never been studied in detail in *TTR* mutation-specific cohorts.

Material and Methods: Serum levels of NfL will be measured five times over a 12-month period in asymptomatic Glu89Gln carriers and stage 1 polyneuropathy ATTRv Glu89Gln patients.Proximity Extension Assay, offered by Olink, combines DNA-based and antibody-based techniques for precise protein biomarker detection. The high-performance Olink Target 96 Neuro Exploratory protein biomarker panel will be used for this analysis.

Results: At the time of writing, 136 samples from 31 symptomatic patients were analyzed, with concentrations of NfL varying between ~1 pg/mL and ~180 pg/mL. The final cohort will consist of ~100 participants (patients and asymptomatic carriers), and all data needs to be collected, in order to be statistically analyzed.

Conclusion: ATTRv is highly prevalent in Bulgaria, which requires reliable diagnostics with practical applicability. The implementation of new reliable biomarkers for the prognosis and monitoring of ATTRv will enable more precise disease management and treatment evaluation.

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P25 Genetic Counselling/Services/Education

P25.001.B Building relationships and collaboration in genomic medicine

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Background: Relationships are foundational to effective implementation and are critical to scale up genomic healthcare. This study explores relational interactions at intra- and inter- professional levels among genetic counsellors, physicians, and other non-genetic health professionals working in innovative models of mainstream genomic care.

Methods: Qualitative interviews were conducted with 29 participants: 8 physicians, 16 genetic counsellors, and 5 nurses/allied health professionals across 7 medical specialties within a publicly funded health system in Australia. Data analysis was guided by Relational Theory and the Theoretical Model for Trusting Relationships and Implementation.

Results: Participants described technical strategies to build rapport and trust via *'frequent interactions'*, such as increasing physical presence in clinics or engaging in corridor conversations about practice. GCs used co-counselling appointments with physicians to *'demonstrate expertise'* in consent discussions and counselling. Co-learning across disciplines was facilitated by relational strategies of *'empathy'* and *'authenticity'*. These strategies applied when participants' were identifying their own knowledge and practice gaps, recognizing the contribution of their colleagues to support them to improve their practice. More knowledgeable or skilled participants showed empathy to colleagues by partnering with them, being willing to share skills and knowledge, and ensuring colleagues were aware of relevant genomics education resources and activities.

Conclusion: Positive collaborations needed to scale up genomic healthcare can be achieved by leveraging technical and relational strategies with the inter-related components of the Model reinforcing one another. Variations in implementation outcomes of mainstream models may be explained by the adoption of these strategies.

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P25.002.C How can clinical genetic services support 'mainstreaming' of genomics? Insights from the 10-year Melbourne Genomics program

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Background: The benefits of genomics are evident across a growing number of medical specialties. For patients to realise these benefits, genomics must be provided within each speciality ('mainstreaming') and not solely through clinical genetics. Melbourne Genomics Health Alliance has run a 10-year systems change program to support integration of genomics into healthcare in Victoria, Australia. Clinical genetic services have played a critical role in the program; as it draws to a close, there is a need to capture practical lessons learnt as a tool for clinical genetic services to inform future mainstreaming efforts in a range of healthcare contexts.

Methods: We took an interpretive description approach to identify how genetic services can support mainstreaming. Data sources from across the program were critically reviewed, drawing on selected models and theories of change (COM-B, diffusion of innovation) and applying a complexity lens. Iterative rounds of synthesis and validation with clinical genetic services are underway to ensure utility of final results.

Results: Two broad models (interdependent, independent) have been identified for delivery of genomic care by other specialist services, along with three types of supports needed for quality care: access to expertise; point of care resources; guardrail mechanism. Influences on model selection and what helps and hinders to embed genomics in medical specialty care are being verified.

Conclusion: Clinical genetic services are crucial in the ongoing 'mainstreaming' of genomics. This synthesis will ensure lessons from a coordinated systems change program can be practically applied in the future across diverse healthcare contexts.

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P25.003.D The integration of genetic counsellors as a priority: a new model for Genetics healthcare provision in Portugal

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Background: In the last decades, technological developments lead to significant advancements in Genetics. In Portugal, serious limitations persist, compromising the functioning of genetic healthcare, including a shortage of trained professionals, limited infrastructure, and inadequate integration of services into routine healthcare. This study aimed to promote discussion among medical geneticists' professionals, to outline concrete actions to address gaps in healthcare provision.

Material and Methods: Three focus groups were conducted with 19 specialists in medical genetics. The data were analyzed using the thematic analysis to extract the main themes from the discussions.

Results: Four conceptual themes emerged: (i) framing Portuguese genetic services within the European context; (ii) improving of medical genetics education and population literacy; (iii) transforming of medical genetics services; and (iv) operationalizing the change. The results demonstrated that enhancing training resources and strengthening multiprofessional teams is viewed as a way to improving the responsiveness of genetic services. The integration of genetic counsellors was identified as a top priority and a visionary step toward improving the overall healthcare system in the country, ensuring a more personalized and accessible service for individuals with genetic conditions. Integrating medical genetics into all specialties and primary care, as well as updating the national network of medical genetics, were also identified as critical areas for improvement.

Conclusion: A new care model with a multidisciplinary and collaborative approach within healthcare teams is necessary in Portugal. The findings have been compiled into recommendations to inform urgent decisions surrounding the implementation of structural changes in Portuguese genetics services.

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P25.004.A Direct letters to relatives at risk of hereditary cancer - A multi-center randomized trial on healthcare-assisted versus family-mediated risk disclosure (DIRECT-study)

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Background: Risk-reducing programs for hereditary breast and ovarian cancer (HBOC) and Lynch syndrome can reduce cancer morbidity and mortality in at-risk relatives. Observational studies suggest that direct contact from healthcare may increase genetic counseling (GC) uptake as compared to family-mediated risk disclosure, but randomized trials are lacking. This randomized controlled trial assessed whether direct contact via letters to at-risk relatives increases GC uptake compared to family-mediated communication alone.

Material and Methods: Between 2020–2023, HBOC or Lynch syndrome patients were randomly assigned to family-mediated disclosure (control) or family-mediated disclosure plus the offer of sending direct letters to at-risk relatives (intervention). The primary outcome was GC uptake within 12 months, measured as the

proportion of eligible relatives at risk contacting a Swedish cancer genetics clinic.

Results: A total of 165 families (median: 4 eligible relatives, range: 1–26) were randomized to control (n = 79) or intervention (n = 86). GC uptake was 67% in controls and 71% in the intervention group ($P = .23$). After adjusting for predefined variables and co-variates, there was still no significant difference between groups (OR 1.24, CI 0.79–1.95, $P = .34$). Distant relatives had lower uptake than first-degree relatives (OR 0.27, CI 0.18–0.40, $P < .001$), while female relatives had higher uptake than males (OR 2.17, CI 1.50–3.12, $P < .001$).

Conclusion: In this randomized controlled trial, GC uptake exceeded 65% in both groups. The intervention of direct letters did not show superiority over family-mediated communication alone.

C.O.I. Disclosure:

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P25.005.B How do genomic multidisciplinary teams foster collaboration, enabling genetic mainstreaming and precision medicine?

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Background: Growing demand for genomics in many subspecialties of medicine has led to massive challenges in service provision, education and upskilling for non-genetics clinicians. The Sydney Children's Hospitals Network established Paediatric genomic multidisciplinary team (MDT) meetings to address this increased demand for genomic testing, and facilitate mainstreaming. This MDT approach has been successful in genomic mainstreaming over 2000 patients since 2020 into paediatrics and subspecialties such as obstetrics, neurology and oncology.

Material and Methods: Mixed methods qualitative research was undertaken to understand the impact of the genomic MDT on adoption of genomics. Guided by the implementation science based Genomic Medicine Integrative Research and Theoretical Domains Frameworks, we designed surveys to understand the barriers and enablers to mainstreaming in the MDTs. Additionally, focus groups were arranged to explore the sustainability and feasibility of the MDT approach.

Results: 33 survey responses, from clinical genetics and 6 paediatric subspecialities, revealed that 74% agreed the MDT facilitated a genetic diagnosis. Most had incorporated MDTs into standard practice, using them for testing, results, and management discussions. Focus groups highlighted cross-disciplinary collaboration and facilitating mainstreaming in a safe environment as key MDT enablers. Sustainability barriers included the lack of administrative support, time, and the need for MDTs to adapt to clinicians' needs as they upskill in genomics.

Conclusion: We have demonstrated the vital role of genomic MDTs in facilitating mainstreaming and upskilling in genomics for non-genetics clinicians. This study adds further qualitative evidence on the implementation barriers and enablers and sustainability issues for long term feasibility.

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P25.006.C 'Choosing the main character'- Healthcare professionals' attitudes towards counselling patients about genetic cancer risk disclosure to relatives. An interview study in the Swedish mainstream testing era

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Background: Healthcare professionals and patients have a shared responsibility for at-risk relatives being informed about hereditary cancer risk. Genetic counselling practices are changing in Sweden as mainstream testing is implemented in cancer services, and the responsibility for relatives is not regulated in guidelines. This study aimed at investigating attitudes towards counselling patients about risk disclosure to relatives among both genetic and non-genetic healthcare professionals handling genetic test results at cancer and clinical genetics services in Sweden.

Material and Methods: A qualitative study with reflexive thematic analysis of fourteen semi-structured interviews with healthcare professionals.

Results: We present the results as four positions that healthcare professionals hold, and move between, depending on their attitudes towards counselling patients about risk disclosure to relatives. The positions vary in terms of if healthcare or the patient is percieved as ultimately responsible for relatives being informed, and if the healthcare professional's focus is on the patient or on the relative. The positions are visualised in a four-fielder: 1. 'The patient is my one and only interest: It´s clear and quite simple'. 2. 'The patient and I are allies: It´s a joint interest'. 3. 'The at-risk relative needs to know, but the patient is in charge: It´s out of my hands'. 4. 'The patient is a mean to reach the at-risk relative: It needs to be done'.

Conclusion: These results could serve as a basis for discussions on roles and responsibilities, moving towards a best practice for genetic risk communication and counselling in the mainstream testing era.

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P25.008.A Unveiling decision-making and family communication of genetic information during and after genetic counseling: Preliminary results from a qualitative prospective diary study

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Australia, [9]European Huntington Association; and Portuguese Huntington Association, Lisboa, Portugal, [10]Faculty of Psychology and Educational Sciences, University of Coimbra, Coimbra, Portugal, [11]CIBIT – Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Coimbra, Portugal, [12]Cardiff UniversityDivision of Cancer and Genetics, Cardiff, United Kingdom, [13]LOHA Health Ltd, London, United Kingdom, [14]ICBAS School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal.

Background: Family disclosure of genetic information is essential to allow at-risk relatives to make informed decisions about health management and life-planning. Yet, existing research is limited and mostly retrospective. Written diaries could provide prospective insight into family communication, exploring the decision-making process in this context. We aimed to explore consultands' experiences, perspectives and decision-making on family communication of genetic information, as they undergo genetic counseling (GC).

Material and Methods: As part of a prospective, multicenter, multi-method qualitative study, we used unstructured written diaries to examine decision-making trajectories related to family communication of genetic information. Participants included consultands undergoing predictive testing for autosomal dominant late onset neurological, cardiogenetic and oncogenetic conditions. From the first GC consultation until four weeks post-result disclosure, consultands recorded (paper or email) their reflections, feelings and decisions on family communication of genetic information. Diaries were transcribed and analyzed using thematic analysis.

Results: Of the 56 participants, 24 returned their diaries totaling 57 entries, which varied per consultand (M = 2.38; R = [1-14]). Preliminary analysis of the diary contents suggests that consultands mainly reported their (a) decision-making process surrounding disclosure, (b) family communication patterns on genetic information, (c) knowledge of the condition and their family history, and (d) daily living with familial risk.

Conclusion: To our knowledge, this study is the first to employ diaries to prospectively explore consultands' decisions on family communication of genetic information. Findings from this study may clarify what influences these decisions regarding family communication and inform the development of resources to facilitate them within GC.

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P25.010.C Exploring individuals' experiences with self-reported unmet need for genetic testing

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Background: In practice, healthcare systems and insurers define "need" for genetic testing based on its potential clinical utility. However, it remains unclear how the public perceives the need for genetic testing and whether this aligns with clinical utility.

Material and Methods: We recruited Canadian participants through a survey distributed through a market research company. Participants who self-reported need for genetic testing on the survey were then purposively sampled to maximize diversity on the dimensions of race, province of residence, gender, and conditions of concern, to complete a semi-structured virtual interview. We used an interpretive description approach and reflexive thematic analysis.

Results: We completed 19 interviews and found that participants' self-identified need for genetic testing was informed by their experiences with genetic information, perceptions that genetic information is actionable (clinical utility) and that it has personal meaning (personal utility). Clinical genetic testing would not have been indicated for most participants based on their personal and family history; however, they had unmet informational and psychological needs, indicating unmet need for genetic counselling. Participants described that unmet expectations for genetic testing contributed to medical distrust and dissatisfaction.

Conclusion: Public understanding of need for genetic testing is complex and varied. Participants described many perceived benefits of genetic testing that are not reflected in how need is operationalized in reimbursement decisions.

C.O.I. Disclosure:

K. Borle: None. **L.D. Lynd:** None. **J. Austin:** None.

P25.011.D The critical missing piece: hospital executive leadership in implementing genomic medicine

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Background: Effective, equitable genomic care requires systemic change, particularly as use of genomics expands beyond clinical genetic services. National strategies address policy and funding, but don't guide hospital executives in making the necessary organisation-wide changes for safe, high-quality care. Without this leadership, implementation will be fragmented and inequitable. No existing frameworks guide the effective use of genomics across a hospital. We aimed to fill this gap by developing a tool to support executive-led genomics implementation.

Methods: Using the Knowledge-to-Action framework, we conducted an iterative design process with 85 hospital executives and genomic experts (14+ organisations). A multidisciplinary group, including clinical-governance experts, developed a maturity assessment tool (MAT) for hospitals. After iterating at a stakeholder workshop, five diverse hospitals tested usability. The MAT was then distilled into key actions for executives, with co-designed resources to support these actions. The resulting 'Genomics and Your Hospital toolkit' is now live.

Results: Usability testing revealed challenges in assessing genomic maturity due to siloed activities, limited awareness of their scale, and a lack of collective approaches. Hospital leaders valued the opportunity to discuss genomics through the 'shared language' provided by the MAT but still needed help prioritising implementation efforts. Therefore, 7 actions for healthcare leaders formed the resulting toolkit which received 400+ views in its first 3 weeks and an average engagement time of 4.5 minutes (benchmark: 70–90 seconds).

Conclusion: Adopted by the Government insurer, the Genomics and Your Hospital Toolkit helps hospitals plan genomic implementation, supporting equity of access across a health system.

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P25.012.A Strengthening collaborative networks between medical school and tertiary care hospitals for equitable diagnosis of rare diseases in rural areas of Thailand

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Background: In rural Thailand, rare disease diagnosis and treatment gaps arise from a shortage of pediatric geneticists. This results in healthcare inequities. A collaboration between Ramathibodi Hospital and Maharat Nakhon Ratchasima Hospital implemented the RARE for 9 and PED-GRIN models. This study evaluates their effectiveness in improving diagnosis and treatment outcomes in rural communities.

Material and Methods: From July 1 to December 31, 2023, we recruited patients under 18. Participants accessed a one-stop service clinic at Maharat Nakhon Ratchasima Hospital, which included genetic testing via whole genome sequencing (WGS). Pediatric geneticists from Ramathibodi Hospital provided supervision and consultation through an online platform (teleconsultation, PED-GRIN model) for WGS interpretation, clinical management and genetic counseling recommendation. Demographic data were recorded, and the benefits were evaluated by comparing the percentage of patients receiving diagnoses and precision medicine.

Results: A total of 266 patients aged 2 months −15 years were included; 80% were diagnosed with uncontrolled epilepsy. Genetic diagnostics were performed on 230 patients using WGS. Abnormalities were detected in 70% of WGS cases, 50% of targeted gene cases, and 16.6% of CMA cases. Precision medicine was administered to 50% of the patients, and the satisfaction rate among patients and caregivers exceeded 98%. Access to diagnosis increased by 30 times compared to the previous period before the establishment of the collaboration, with a diagnostic yield comparable to international studies.

Conclusion: The collaborative pathway enhances equity for patients with rare diseases in rural areas by improving access to timely diagnosis and effective treatment.

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P25.013.B Unique Experiences. The lived experience of those affected by rare chromosome or gene disorders

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Background: Unique (a UK-based charity working internationally) provides support, information and advocacy to individuals and their families/carers affected by rare chromosome or gene disorders. A survey conducted May/June 2025 sought insights into the day-to-day lived experience of those affected by these rare conditions.

Material and Methods: An online survey consisted of a mix of open text, 5-point scales and multiple choice was shared via email and Unique's social media channels. The responses were anaylysed by both statistics and thematic analysis.

Results: 2,154 people completed the survey. The majority of respondents (81% / 1731) were mothers/stepmothers caring for someone with a rare chromosome or gene disorder suggesting a potential bias towards the maternal caring maternal caring experience. 5% (111) were from affected individuals. Lack of professional awareness, low public understanding, and struggles to access appropriate information and services results in high levels of anxiety (70%,1253/1801), frustration (60%, 1073/1801) and stress (60%, 1086/1801). Having, or caring for someone, with a rare chromosome or gene disorder can strengthen family relationships and bonds, however, living with constant uncertainty, and the complexities of care and managing the condition, often also impacts social networks and relationships resulting in feelings of isolation and loneliness.

Conclusion: Living with or caring for someone with a rare chromosome or gene disorders can create significant challenges all areas of life and life chances which places strain on individuals and families. However, families and individuals often become experts in their rare chromosome and gene disorder and are determined and powerful advocates.

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S.L. Wynn: None. **L. Roberts:** None. **C. Wilmshurst:** None.

P25.014.C A bespoke therapeutic counselling service for the Fragile X community

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Background: Fragile X syndrome and associated conditions present unique emotional and psychological challenges for individuals and their families. Standardized counselling services often lack the specific genetic and psychosocial understanding required, leaving clients to spend significant time explaining Fragile X rather than focusing on their emotional needs. To bridge this gap, a bespoke therapeutic counselling service was developed in collaboration with the Fragile X Society. This service integrates genetic counselling with person-centred therapeutic support, ensuring that clients receive informed and empathetic care tailored to their lived experiences.

Material and Methods: The service, launched in August 2023, is led by a trained genetic counsellor and registered therapeutic counsellor (BACP). Sessions are delivered remotely via Zoom or telephone, making the service accessible across the UK. Clients include individuals with Fragile X syndrome, premutation carriers, and relatives. The service provides up to six 50-minute person-centred counselling sessions, focusing on emotional processing, adjustment, and coping strategies.

Results: Since inception, 16 individuals have accessed the service, each receiving an average of six sessions. Feedback has been overwhelmingly positive, with clients reporting significant improvements in anxiety, self-awareness, and relationships. One client noted, "For the first time in a long time, I know that it is possible for me to live a happy life not dictated by anxiety."

Conclusion: This innovative service demonstrates the need for integrated genetic and emotional support in rare disease communities. Challenges, including non-attendance and policy adaptation, are being addressed to ensure sustainability. The success of this model highlights the value of specialized therapeutic genetic counselling services.

C.O.I. Disclosure:
V. Arthur: None.

P25.015.D Team Based Care Model for Evaluating and Counselling of Neurodivergent Children with Chromosomal Copy Number Variants of Uncertain Significance

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Background: Chromosomal microarray has been used as first-line genetic test for children with autism spectrum disorders (ASD), intellectual disability (ID), and developmental delay (DD). Variants of uncertain significance (VUS) are common findings. Despite the challenge with interpreting VUS, parents of children with ASD/ID/DD often value receiving these results and appreciate genetic counseling regarding their implications.

Material and Methods: A retrospective review was conducted on 177 charts of children seen at a general Genetics clinic in Toronto, Canada, between January 2013 and June 2022 with ASD/ID/DD and a microarray finding of VUS. We assessed the clinical impact of VUS findings and implemented a novel service delivery model involving a pediatrician and genetic counselor (GC) team. Patients with dysmorphic features or complex findings were referred to a geneticist for further evaluation and testing.

Results: Among the cases reviewed, 23.7% (42/177) had CNVs susceptibility locus for ASD/ID/DD, altering recurrence risk counseling. An additional 14.7% (26/177) were carriers of autosomal recessive conditions. Actionable follow-up recommendations, including specialist referrals, imaging, and laboratory tests, were made in 25.4% (45/177) of cases, with clinical or personal utility identified in 63.8% (113/177). Of the patients evaluated via the pediatrician/GC model (51.4%, 91/177), only 4.4% (4/91) required geneticist evaluation. High satisfaction was reported with the model in both in-person and virtual settings.

Conclusion: This is the first study to document the management impact of VUS on microarray in children with ASD/ID/DD. The pediatrician-GC team model is effective, enhances service delivery, and integrates genetics into primary pediatric care.

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P25.016.A Cumulative Cost Analysis of Prevention versus Treatment in CDH1-related Hereditary Diffuse Gastric Cancer (HDGC): A Seven-Country European Study

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Background: *CDH1* inactivation causes Hereditary Diffuse Gastric Cancer (HDGC), characterized by early-onset gastric (GC) and breast cancers (BC). While intensive surveillance and risk-reduction gastrectomy and mastectomy prevent disease progression, the cumulative costs of HDGC healthcare management remain unexplored.

Methods: We analyzed 412 HDGC-*CDH1* cases from seven European countries (Portugal, Spain, France, Norway, Sweden, the Netherlands, Germany) from 2000–2023, including 232 pathogenic variant carriers (96M:136F) and 180 negative family members. The HDGC care pathway, developed through the PREVENTABLE EU project ((www.preventable.eu), includes specialized care routes, clinical trajectories, and healthcare costs. Cumulative cost data were calculated using Portugal's healthcare system pricing lists.

Results: Of 232 carriers, 172 (74%) followed prevention routes, while 60 (26%) required treatment due to 24 early-onset cancers (8 breast, 14 stomach, 2 both) or 36 advanced cancers (12 breast, 23 stomach, 1 both). Seventeen carriers died (15 stomach, 2 breast cancer), with median follow-up of 43 months for prevention, 76 for early-onset, and 24 for advanced cases. Prevention costs averaged €4,959 (range: €142-€28,533), while early and advanced cancer treatments were 2.7-fold (€13,456; range: €1,297-€26,121) and 4-fold higher (€20,130; range: €3,800-€71,256), respectively. Monthly costs for early (€1,087) and advanced cancer treatment (€1,226) were approximately 6-fold higher than prevention (€186). Total cumulative costs: €853,008 for prevention (172 patients) versus €1,047,615 for treatment (60 patients).

Conclusion: This first European cost analysis demonstrates that HDGC treatment costs exceed prevention costs, even when prevention covers a 3-fold larger population, providing economic evidence supporting early identification and preventive care for *CDH1* carriers.

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Bajalica-lagercrantz: None. **K. Verbeek:** None. **R. Sousa:** None. **A. Azevedo:** None. **C. Oliveira:** None.

P25.017.B Response factors for declining to participate in a large-scale genomic study: the potential role of the primary care physician in improving study participation

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Background: The future of medicine relies on individualized care based on the patient's genomic and exposomic factors. Despite the promise of genetic screening in improving health outcomes, many patients are hesitant to participate in genomic research. Primary care physicians (PCPs) play a crucial role in educating patients about genomics. This study aims to understand why patients decline participation in a large-scale study offering whole exome sequencing.

Materials and Methods: From January 2020 to May 2024, patients over 18 years old were invited via electronic messaging to participate from Mayo Clinic's campuses in Minnesota, Florida and Arizona. The study aimed to reach at least 100,000 consented participants. Those who declined were asked to select specific reasons and/or provide additional responses in free text. Data collected included age, gender, race, ethnicity, rural/urban area, and whether the patient had a PCP.

Results: Of the first 545,196 invited participants, 38,088 consented (64.7% women) and 12,705 actively declined (57.4% women). Those actively declining were 90.1% white and an average age of 61 years. Patients with a PCP were more likely to cite Electronic Health Record concerns and discomfort with research as reasons for declining ($p < 0.01$), suggesting that discussions with PCPs could address these barriers. Lack of a PCP was associated with a perception of no benefit in participation ($p < 0.01$). Older patients perceived less benefit from participating ($p < 0.01$).

Conclusion: While understanding the human genome holds great promise, barriers to research participation exist. PCPs can play an important role in patient education.

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P25.018.C Strategic Development of Genetic Counselling in D-A-CH: Insights from the new German Society of Human Genetics Commission for Genetic Counsellors

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Background: In June 2024, the German Society of Human Genetics (GfH) established a Commission for Genetic Counsellors (GCs) with 86% member approval, marking a significant step for the genetic counselling profession in German-speaking countries (Germany, Austria, and Switzerland; D-A-CH). This specialized committee aims to advance the genetic counsellor profession, influence policy, and drive improvements in the field.

Material and Methods: An online workshop was conducted to define the commission's key goals, involving 85 participants from genetic centres across D-A-CH, including a comprehensive representation of German-speaking GCs and medical geneticists. The event comprised introductions of commission members, presentations, and open discussions engaging participants in a structured process to identify key priorities for future initiatives.

Results: The GfH GC commission's primary goal is to create the prerequisites for the legal recognition and effective integration of GCs into clinical-genetic services in the D-A-CH region. Responding to the increasing need for more trained professionals, standardized education aligned with the European core curriculum and internationally defined responsibilities and competencies for GCs. Seven major goals were defined and reflected in the statute of the GfH Commission for GCs.

Conclusion: Through a collaborative, international workshop involving a diverse group of GCs and medical geneticists from the D-A-CH region, the commission successfully defined strategic goals for its work. This consensus-driven approach identified the most critical requirements for the full integration of GCs into medical genetic services across the D-A-CH countries, providing a robust framework for future development and implementation of the GC profession.

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P25.019.D Polygenic risk in practice: An evaluation of genetic health care providers' learning needs and preferences

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Purpose: Polygenic risk scores (PRS) provide an estimate of the genetic contribution to health conditions. Genetic health professionals (GHPs) report they do not have the knowledge and skills required for using PRS, representing a barrier to testing implementation. This study aimed to explore GHP learning needs and identify strategies to promote engagement in PRS education.

Methods: Australian and USA-based GHPs with prior experience in PRS use were eligible to participate in focus groups/interviews ($n = 30$), which were transcribed and analysed using content analysis. Interview guide and collected data were mapped to the COM-B model of behaviour change to identify target areas for intervention.

Results: All GHPs felt motivated to pursue PRS education due to a belief it would optimise patient care and ensure they met professional standards. Reported learning needs included key PRS concepts, result interpretation and effective patient communication. However, there was variability in the amount of PRS knowledge that GHPs felt was necessary to integrate testing into clinical practice, highlighting the need for modular programs to

address specific learning needs. GHPs reported that PRS training opportunities and protected time for attendance would increase capability. A need for clinical guidelines was identified as an area of priority to facilitate GHPs' use of PRS. Opportunities to support PRS use included quick reference resources, embedded practice models such as multidisciplinary teams and strategic support.

Conclusion: Our data highlights the complexity of PRS education and will be used to support delivery of targeted PRS educational materials as part of a broader implementation strategy.

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P25.020.A Navigating the unknown: a qualitative study of parental responses to a prenatal genetic mosaicism result

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Background: Prenatal detection of placental mosaicism challenges genetic counseling, as predicting fetal involvement and clinical impact is difficult. This creates a complex situation for expectant parents, who must manage uncertainty and waiting time for diagnostic results and pregancy outcomes. This study explores the experiences and counseling needs of parents who were prenatally diagnosed with confined placental mosaicism and chose to continue pregancy.

Material and Methods: An exploratory, qualitative study design was used based on semi-structured interviews. Participants were recruited from two Departments of Clinical Genetics. Fourteen interviews were conducted six months postpartum, recorded, and transcribed verbatim. Data were analyzed using reflexive thematic analysis.

Results: The diagnosis of confined placental mosaicism introduced unexpected uncertainty, significantly impacting the parents' daily lives. Following genetic counseling, many struggled with the lack of clear answers. Nonetheless, most found the consultation reassuring, offering hope despite the ongoing uncertainty, which included worry about the future, fear of losing the child or undergoing a late-term abortion. During pregnancy, parents used coping strategies, such as avoidance or seeking support from their closest relationships. After birth, worry decreased remarkably. However, two couples decided not to have more children, fearing they might face a similar experience again.

Conclusion: A diagnosis of confined placental mosacism creates uncertainty among parents, emphasizing the need for specialized genetic counseling. At the time of the conference, specific suggestions for enhancing genetic counseling will be presented.

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P25.021.B Collective decision process on counselling for recessive syndromes in genes predisposing to solid tumors

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Background: The Catalan Guidelines on Hereditary Cancer Predisposition Syndromes in Non-Hematologic Adult Cancers recommends offering reproductive counseling (RC) to individuals carrying a germline pathogenic variant (gPV) in cancer predisposition genes. This study describes the decision-making process regarding RC for recessive syndromes (RS) in genes predisposing to solid tumors.

Material and Methods: The Delphi method was used to survey about RC on RS associated to solid-tumour susceptibility genes. A coordinating group analyzed PV prevalence, offspring risk, syndrome severity and international and national guidelines recommendations. A multidisciplinary panel of 64 experts working in hereditary cancer in Catalonia's public healthcare system was summoned to answer several rounds of REDCap-based questionnaire, with statements accepted at ≥75% consensus.

Results: After two consultation rounds and a validation joint meeting, the agreements were:

- Monoallelic gPV carriers should receive RC on the RSin families with affected members of the RS or consanguinity.
- Carriers of gPVs in *MUTYH*, *ATM*, *BRCA2*, and *PALB2* should be informed of the recessive syndrome and offered reproductive counseling.
- Counseling on RS is not recommended for syndromes associated with genes with a prevalence <1/250 unless family history is indicative.
- Genetic testing for the partner will be offered to biallelic *MUTYH* gPV carriers. A decision remains pending in the third round regarding extending this offer to carriers of gPVs in ATM, BRCA2, and PALB2 based on family history.

Conclusion: RC is recommended for monoallelic carriers with RS affected family members or consanguinity. However reproductive counseling should be also offered to carriers of most prevalent genes.

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P25.023.D Assessing knowledge gaps and educational needs on rare diseases among medical students, healthcare trainees, and physicians: a national survey

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Background: Rare diseases, which have a genetic etiology in 80% of cases, are often underrecognized in medical education. This study aimed to assess the knowledge of rare diseases among medical students, health science students, and physicians, and to identify their educational needs in this area.

Material and Methods: The survey involved 163 medical students, 248 healthcare science students, and 393 physicians, including 52% specialists, 30% dentists, and 18% medical residents. The survey assessed participants' understanding of rare diseases, awareness of key facts, and educational preferences.

Results: The survey revealed significant knowledge gaps. While 90% of respondents had heard of rare diseases, only 15% could define them or estimate their prevalence. Physicians significantly underestimated their frequency, with more than half believing they occur less than 1 in 10,000 individuals. Limited awareness was also noted regarding the genetic origins of rare diseases: only 46% of healthcare science students and 69% of medical students knew the most common cause is genetic. Both groups reported low confidence in their preparedness to manage rare disease patients—only 13% of medical students and 25% of healthcare science students felt adequately trained. Over 90% of respondents supported rare disease education in curricula, with most favoring expert-led lectures and case-based learning.

Conclusion: The findings highlight significant knowledge gaps and a strong demand for enhanced education on rare diseases in medical and health science curricula. The results will guide the development of targeted educational curricula for medical students and healthcare professionals.

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P25.024.A artificial intelligence-simplified information to advance reproductive genetic literacy and equitable care

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Background: Reproductive genetic testing and counseling holds the potential to support a personalized approach to managing genetic disorders. However, low health literacy and complex medical information often hinder patient understanding, leading to over- or under-use of testing. This issue is raising concerns that the fertility industry exploits patient vulnerability for profit. To address this challenge, a bottom-up approach is necessary to empower patients with accessible and sufficiently accurate information to attain equitable care.

Material and Methods: We conducted a comparative observational study to assess the performance of four large language models (LLMs) in simplifying patient education materials (PEMs). We collected thirty PEMs on six topics from authorized websites and simplified them using a consistent prompt. We then measured readability improvements using seven standardized metrics and assessed the accuracy of the simplified texts by experts (n = 30).

Results: LLMs significantly enhanced the readability of patient materials. Although Gemini yielded greatest readability improvements, reducing text complexity to the recommended readability standards for PEMs, GPT-4 demonstrated the highest accuracy by preserving essential medical information. These findings emphasize the need to balance accessibility with content integrity, as excessive simplification may lead to omission of critical health information.

Conclusion: AI-driven tools can bridge health literacy gaps and improve equitable access to reproductive care. Their integration into patient education may enhance informed decision-making, reduce healthcare disparities, and alleviate the communication burden on providers. However, careful evaluation of AI-simplified PEMs is required to ensure simplification does not lead to critical omissions, which may result in patient dropout from critical testing.

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P25.025.B Developing a medical visualisation approach for innovative teaching materials in clinical genetics

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Background: Teaching of genetics for healthcare has to be accessible to individuals from a range of backgrounds with diverse learning styles, where traditional text-based materials may be insufficient. Simultaneously, there is a greater requirement for genetic knowledge for providers of healthcare at different levels.

Materials & Methods: We used a novel illustrative approach to create genetic/genomic learning resources. Our approach drew on Medical Visualisation techniques and has considerable commonality with the use of illustrated abstracts and infographics. We used large numbers of illustrations supported by concise text and creative analogies to enhance engagement. A translation table was built to expedite translation into any other European language.

Results: Our open access illustrated guide to basic concepts in clinical genetics (https://discovery.dundee.ac.uk/en/publications/berg-and-laws-survival-guide-to-genetics), targeted at medical students, has also proved of interest to a wider audience of genetics healthcare professionals, including genetic counsellors and specialist doctors in training. It has been adopted by the APOGeE project as a general resource, with translation into French. Initial feedback from a cohort of undergraduate medical students showed that all respondents felt more confident in their understanding (75% definitely more confident, 25% somewhat more confident). 100% of students felt that the subject was covered in the right depth.

Conclusion: Our medical visualisation approach to genomic learning resources shows promise for future development. A second resource for genetics counselling is near completion, and has allowed use of techniques inspired by graphic novels. This allows depiction of complex and hard to teach counselling concepts in a highly innovative fashion.

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P25.026.C Navigating Genetic Counselling and Cancer Risk in Transgender and Gender-Diverse Patients

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Background: Transgender and gender-diverse (TGD) individuals face unique challenges in genetic counselling, particularly regarding cancer risk assessment. Existing screening guidelines do not adequately consider gender-affirming treatments, leading to gaps in risk stratification. This case highlights the complexities of counselling a transgender man with erythropoietic protoporphyria (EPP) with a family history of breast cancer, who underwent gender-affirming top surgery requiring testosterone therapy.

Material and Methods: A 30-year-old transgender man (assigned female at birth(AFAB)) with clinically diagnosed EPP was assessed for hereditary cancer risk given a strong maternal family history of breast cancer. Risk stratification was conducted using CanRisk, incorporating his gender identity, hormone therapy, and prior top surgery. The case was reviewed against current international guidelines on genetic counselling for TGD individuals.

Results: Genetic analysis confirmed likely pathogenic in-trans FECH variants, supporting the EPP diagnosis. This case exemplifies the challenges of applying standard risk models to patients whose risk-profiles are altered by gender-affirming interventions. CanRisk classified the patient as moderate risk for breast cancer based on AFAB status, though it did not account for top surgery. Given potential residual breast tissue and the uncertain impact of testosterone, counselling emphasised self-examination and physician follow-up.

Conclusion: This case highlights the urgent need for gender-inclusive genetic counselling guidelines and the development of personalised risk assessment models that incorporate gender-affirming treatments. Integrating these considerations into clinical practice, and refining risk models through further research is essential to improving care and reducing healthcare disparities among TGD individuals.

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P25.027.D Integrating rare variants and PRS in breast cancer prediction and patients counselling

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Background: Advances in Polygenic Risk Scores (PRS) have shown potential for improving risk prediction. However, the combined contribution of rare germline variants and PRS to breast cancer susceptibility remains largely underexplored.

Material and Methods: Exome sequencing and genome-wide PRS assessment on 211 female cancer patients. Stratification into four risk categories for rare variants: i) high, ii) medium iii) low, iv) no P/LP variants in known cancer driver genes, and three risk categories for PRS: high (10 percentile), low (90 percentile) and intermediate. Odd ratio (OR) combination was calculated on UK biobank.

Results: The integration of combined risk assessment improved patients counselling, especially for invasive decisions such as bilateral mastectomy and oophorectomy. For example, the most *BRAC1/2* mutation carriers fell in the intermediate PRS having an OR of 4.52 (95% CI: 3.8–5.37, $p < 0.001$), while one fell in the high PRS raising OR to 11.47 (95% CI: 9.62–13.67, $p < 0.001$) and three in the low PRS having an OR of 1.68 (95% CI: 1.41–2, $p < 0.001$). Even more interesting, *ATM/CHEK2* intermediate risk variants patients falling in high risk PRS reached OR of 6.58 (95% CI: 5.52–7.85, $p < 0.001$) which is higher than *BRCA1/2* with intermediate or low PRS and to whom surgery should be suggested.

Conclusion: Overall, our results demonstrate that integrating PRS and rare variant analysis significantly enhances patient stratification and appropriate counselling in breast cancer. Moreover, they lay the groundwork for the development of new guidelines for more personalized genetic counselling.

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P25.028.A Consensus European strategies for ensuring quality genetic counselling in cancer prevention and care

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Background: Genetic counselling is critical to the integration of genetics into cancer care, facilitating informed patient decision making and more accurate diagnoses. However, new strategies are needed to guarantee equitable access to counselling throughout the European Union (EU) - given current healthcare workforce shortages and increasing demands for genetic testing, genetic counselling risks becoming a bottleneck constraining the integration of genetics into cancer prevention and care. This project aimed to identify strategies for addressing common European literacy, workforce, and reimbursement barriers to genetic counselling identified in prior work.

Material and Methods: Genetics, oncology, and patient stakeholders from all EU Member States were invited to an online Delphi survey. Over two phases, participants rated 19 strategies proposed by the research team and other participants according to their Importance, Urgency, and Feasibility and selected their top three priority strategies.

Results: 77 individuals (28 geneticists; 14 oncologists; 18 genetic counsellors; 16 patient representatives; 1 qualified 'other') participated (62% response rate), with each Member State represented. Five strategies met pre-defined consensus thresholds and received a clear plurality of priority ratings: 1) EU-wide genetic counsellor recognition; 2) Including genetics expertise in oncology guideline creation; 3) Shared legal EU genetic counsellor registration/education; 4) Mandatory genetics in oncology fellowship/continuing education; 5) Mandatory counselling reimbursement when clinical guidelines are met. Genetic counsellors rated

solutions #1 and #3 more favorably than geneticists and oncologists ($p < .03$).

Conclusion: The five priority strategies provide clear directions for future European action. Upcoming and recently started EU actions will pilot their implementation.

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P25.029.B Establishing a joint Nordic master's program in genetic and genomic counselling: The Nordic network in genetic and genomic counselling (NNGGC) initiative

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Background: The Nordic Network in Genetic and Genomic Counselling (NNGGC) is a collaboration involving educational and clinical institutions from Denmark, Finland, Iceland, Norway, and Sweden. The network aims to advance education and awareness in the field addressing the growing need for trained genetic counsellors.

Objective: The goal is to establish a joint, quality-assured Master's program in genetic and genomic counselling. The program will train professional genetic counsellors, considering the unique contexts of each Nordic country, and strengthen multidisciplinary and enhance higher education collaboration.

Methods: The program will build on an EBMG-accredited program established at Linköping University in 2021, integrating clinical and academic training with University Hospitals. The curriculum will join expertise and resources, offering diverse learning experiences and research opportunities. A significant portion of the program will be delivered online, supporting the UN Agenda 2030. The program will be primarily taught in English, adding native language practical components in practical experience.

Results: Key planning and implementation activities include funding applications to the Nordic Ministry, establishing agreements, identifying stakeholders, and managing the implementation process. Notable events include on-site meetings in 2024 in Stockholm and in Copenhagen to work on planning for funding.

Conclusion: This Nordic collaboration aims to establish a quality-assured joint Nordic Master's program in genetic and genomic counselling. The initiative aims to increase the number of EBMG-accredited genetic counsellors, promote awareness and growth of the profession in the Nordic region, and provide a basis for future legal recognition, leading to enhanced patient safety.

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P25.030.C Preconception carrier screening tests for X-linked disorders in racially and ethnically diverse community: Challenges to culturally competent screening and counseling

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Background: Preconception carrier screening tests (PCSTs), particularly for X-linked disorders, pose unique challenges when offered to ethno-cultural minorities, due to cultural norms, accepted religious practices, and possible labeling of the female partner. Israeli PCSTs, including fragile-X carrier testing, are sponsored by the Ministry of Health to all citizens. Our aim is to assess the efficacy of standard fragile-X PCSTs among the Jerusalem Arab community, by studying the genetic counseling (GC) process and the rate of prenatal diagnosis (PND) and terminations of affected pregnancies.

Material and Methods: We performed a retrospective analysis on a cohort of Arab women who presented for GC upon identification as *FMR1* carriers (56–150 repeats) between 2010–2022.

Results: Overall, 32 women received GC (aged 17–42), nine of which under the age of twenty-one. All were married except one who was engaged. Sixteen (50%) were pregnant while receiving their results (10–27 weeks of gestation). Three woman had sons with intellectual disabilities. Ten women (31%) chose to perform fragile-X PND: three underwent PGT, four CVS and three AC, in which two affected fetuses were detected, and their pregnancies were terminated. Their genetic counselors felt that many of these patients were not aware of the unique characteristics of the test before taking it and were overwhelmed with the ramifications for themselves, their current/future pregnancies, and marital relationships.

Conclusion: Low uptake of prenatal genetic testing reveals the limitations of PCSTs for a specific community. Implementation of culturally competent screening tests and GC will allow an informed decision for every women/couple.

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P25.031.D Understanding public attitudes toward cascade testing. A Mixed-Methods Study

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Background: Although detecting genetic predisposition to severe conditions offers the opportunity for personalized prevention in family members, only a minority of at-risk relatives seek cascade counselling and testing. This study aims to explore public perspectives and needs, to understand barriers limiting cascade testing uptake.

Material and Methods: A convergent parallel mixed-methods design was used. A questionnaire exploring knowledge and attitudes regarding cascade genetic testing was developed and assessed through cognitive interviews. An improved online

version based on interviews results was then disseminated through social media.

Results: Cognitive interviews revealed four key challenges in understanding genetic information: (1) difficulties with genetic terminology and scientific jargon, causing anxiety and reducing comprehension; (2) ambiguities about "genetic testing" and "family," leading to confusion; (3) misinterpretations of "genetic risk" as a diagnosis rather than a probabilistic concept; and (4) conflation of "authorization" and "responsibility" in communication, exacerbated by privacy concerns. Iterative testing improved the questionnaire's clarity and reliability.From 11/11/2024 to 18/01/2025 N = 576 individuals responded; 92.7% expressed interest in knowing their genetic risk. Individuals willing to receive information showed higher knowledge levels (score: 3.39 ± 0.84 vs. 2.93 ± 0.96, $p = 0.005$). While 72.5% would like to be informed by healthcare professionals of any genetic condition in their family, only 44.6% would delegate this responsibility if they were the probands diagnosed with the inherited condition. Analysis of the factors influencing attitudes toward cascade testing is ongoing.

Conclusion: Genetic terminology should be adapted to meet the information needs of the public; improving knowledge may help promote adherence to cascade testing.

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P25.033.B Assessment of genetic education needs to implement a mainstream process in a tertiary paediatric hospital in Italy

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Background: Understanding genetics is crucial for healthcare providers to effectively manage data and ensure high-quality patient care, in particular in such a rapidly evolving scenario. Global assessment of deficiencies in genetic training, both in formal education and continuing education, has prompted various countries to take action.

Material and Methods: In this study, conducted in a paediatric tertiary hospital in Italy, a questionnaire was sent to non-genetic clinicians. Data is now being gathered on nurses and obstetricians. The aim is to assess readiness to engage with genomic information, interest in further training, insights on existing procedures and attitudes towards mainstream clinical practice.

Results: In total, 123 medical doctors and, as of now, 45 obstetricians and nurses responded. On average, responders did not feel entirely confident in handling genetic topics, education received was perceived as inappropriate to manage such information, 93% of responders reported wanting to participate to genetic educational activities and the majority of them were interested in all topics proposed. A guideline governed mainstream approach to genetic testing was perceived as necessary.

Conclusion: Consistent with findings from other countries, our study revealed a significant knowledge gap. The study on this sample of Italian practitioners showed the general inappropriateness of formal genomic education, the will to participate to training activities and the receptivity for mainstream implementation. Our goal is to facilitate the acquisition and application of genetic knowledge in everyday medical practice, ultimately improving patients' understanding of their conditions and empowering them to make informed decisions.

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P25.034.C Do digital tools support a diversity of patient values? A qualitative study using the Genetics Adviser

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Background: Digital health applications can engage patients in shared decision-making in accordance with their values. Despite the well-established role of patient values in shared decision-making, little is known about the range of values held by diverse populations and how they inform decision-making for genomic testing, particularly for value-ladened decisions about learning secondary or incidental findings (IF) from exome sequencing (ES). Aim To explore the range of values raised when deciding upon IFs to receive from ES and how these values are supported by the Genetics Adviser Methods We conducted semi-structured qualitative interviews with participants undergoing ES and choosing which IFs to receive as part of the Genetics Adviser RCT (Trial registration number: NCT04725565, PMID: 35487723). Interviews were analyzed using interpretive description methods Results Decision-making was described as predetermined and occurred within a 'black box' before using the Genetics Adviser. This 'black box' describes a complex system of motivational factors involved in decision-making (Fig 2). Three overarching values emerged from unpacking this 'black box', including: a sense of family stewardship, an information imperative, and preserving agency (Fig 3). These values spanned multiple themes. Participants appreciated using Genetics Adviser to confirm their predetermined decision by clarifying, contextualizing, and instilling confidence in their decision.

Conclusions: Participants entered decision-making with a predetermined decision guided by three core values, which were supported by the Genetics Adviser. These findings challenge the dogma surrounding decision support and genetic counselling in clinical genetics and can inform how digital tools can better support patient values.

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P25.035.D Systemizing Genetic Health Delivery: A Virtual Clinic Model for Expanding Access to Remote Areas in Saudi Arabia

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Background: Access to specialized genetic services remains a challenge in remote areas, particularly for rare diseases. To address this, we established a virtual genetic clinic in 2021 under SEHA Virtual Hospital, the world's largest virtual hospital, officially recognized by Guinness World Records in 2024. This clinic provides equitable access to genetic counseling, metabolic services, and prenatal diagnostics across Saudi Arabia. We present the results of the first 100 patients, service challenges, solutions, and outcomes.

Methods: The virtual clinic integrates multidisciplinary services, including clinical genetics, metabolic clinics, genetic counseling, dietetics, and advanced genetic testing. To overcome logistical barriers, we implemented home-visit services for sample collection and medication delivery. Telemedicine platforms were adapted to low-bandwidth settings, with local providers facilitating patient engagement. The national newborn screening program's results were integrated, offering a hotline for positive cases, and general dietitians were trained to support metabolic care remotely.

Results: The clinic serves 120 patients per month, with a 55% diagnostic yield for positive genetic cases. Among the first 100 patients, 32.2% were from the West, 24.4% from the North, and 13.3% from the Central region. The South (11.1%) and East (7.8%) were underrepresented, likely due to limited local genetic testing. Challenges such as poor internet connectivity and cultural hesitations were addressed through home visits and telemedicine.

Conclusion: This virtual clinic has significantly improved genetic healthcare access, reducing travel burdens and enhancing patient outcomes, demonstrating telemedicine's potential to improve healthcare equity.

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A.M. Al Hashem: None. **M. Alshehri:** None.

P25.036.A It's all about the VOUS: inconclusive prenatal CMA results, and the role of the genetic counselor

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Background: On 01/02/2019 Chromosomal Microarray Analysis (CMA) replaced Karyotyping as the standard prenatal genetic test in Israel. As a result, genetic counselors and their counselees needed to contend with variants of unknown significance (VOUS). If no sonographic fetal structural malformations are detected, most medical centers in Israel allow counselees to decide whether they want VOUS to be reported, preventing dilemmas caused by such results. The purpose of this study is to estimate the frequency of VOUS in prenatal CMA, and to evaluate factors influencing counselees' decisions.

Methods: A 6-year retrospective analysis (02/2019–01/2025) of 1,883 prenatal CMA samples was performed regarding test indication (advanced maternal age or personal choice), medical center (two institutes), and genetic counselor identity (15 counselors).

Results: A VOUS was detected in 7.6% (143/1,833) of tested samples. Most counselees (1,042/1,883) preferred VOUS not to be reported (55% vs. 45%, $p < 0.00002$). Of the three above variables tested, only one significantly influenced this decision. Depending on counselor identity, a range of 37–90% of counselees refrained from including VOUS in their results.

Conclusion: Despite rapid progress in the ability to accurately classify CMA variants, many variants are still classified as VOUS. To alleviate additional uncertainty and stress in pregnancies, medical centers allow counselees to decide whether to receive uncertain results or not. Our study showed that counselees tend to opt out of receiving VOUS. In our cohort, of the factors tested, counselor identity was the only one to influence the counselee's decision, emphasizing the importance of genetic counseling.

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P25.037.B Genetic Counselling and Information in Autism and Related Psychiatric Conditions: A Swedish Clinical Trial

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Background: Autism is highly heritable and often co-occurs with attention-deficit hyperactivity disorder (ADHD), anxiety, and depression. Limited knowledge exists on communicating genetic information related to autism and co-occurring conditions to autistic individuals, and its impact. This observational clinical study (NCT06740760) evaluates whether providing genetic information on autism and related psychiatric disorders enhances understanding and empowerment in autistic adolescents and young adults.

Material and Methods: A total of 60 autistic participants have been included and randomized into two active groups: one receiving a 1-hour genetic counseling session with a clinician and the other receiving the information via leaflet. The main outcome measure is the Modified Genetic Counseling Outcome Scale-24 (mGCOS-24). Secondary outcome measures include Generalized Anxiety Disorder-7 (GAD-7) and Patient Health Questionnaire-9 (PHQ-9). For those with earlier genetic data available (1), polygenic scores (PGS) for autism, ADHD, depression, and anxiety have been calculated and will be communicated as a percentile range in comparison to two population cohorts.

Results: We have finalized the recruitment, and 42 have completed the study. The main demographics are presented in Table 1. The average percentiles of PGS within the study individuals were 70 for autism PGS, 63 for ADHD PGS, 68 for

anxiety PGS, and 32 for depression PGS. We will present the results from the trial outcomes.

Table 1. Demographic information on study participants.

	Genetic counseling group (n = 33)	Genetic information leaflet group (n = 31)	P-value
Men	17 (52%)	16 (52%)	NS
Women	16 (48%)	15 (48%)	NS
Age in years (mean, range)	24 (16–30)	25 (19–30)	NS
Individual genetic results	16 (48%)	15 (48%)	NS
Occupation			
Employed	11 (33%)	14 (45%)	NS
Student	15 (45%)	11 (35%)	NS
Neither a student nor employed	11 (33%)	8 (24%)	NS
Healthcare contacts			
General practice	8 (24%)	8 (26%)	NS
Psychiatric outpatient clinic	24 (73%)	12 (39%)	P < 0.05*
Additional diagnoses			
ADHD	21 (64%)	19 (61%)	NS
Depression	11 (33%)	9 (29%)	NS
Anxiety	11 (33%)	8 (26%)	NS
Genetic condition	4 (12%)	2 (6%)	NS
None	10 (30%)	12 (39%)	NS

Conclusion: This study will provide insights into the impact of genetic information on the understanding, well-being, and empowerment of autistic adolescents and young adults, potentially guiding future clinical practices.

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P25.038.C The VEDOCO-study: Attitudes towards and experiences with voluntary insurance and cardiac and oncological predictive genetic testing in the Netherlands

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Background: Dutch insurers are, under circumstances, allowed to use genetic information when offering a life or disability insurance. Concerns about the impact of a positive genetic test on obtaining these insurances are often mentioned as a barrier for at-risk relatives (ARRs) to seek genetic counselling and predictive testing. We therefore assessed ARRs' expectations and the experiences of carriers with insurance applications.

Material and Methods: We conducted a cross-sectional retrospective survey study in five genetic centers. Adult ARRs of probands with inherited cardiac or oncological conditions who received genetic counselling between Dec 2016 and July 2022 were invited to complete an online survey.

Results: The survey was completed by 283 of 2359 (12%) invited participants (mean age 50.4 years (SD 14.5); 54% female) from families with inherited cardiac (48.9%) and oncological (51.1%) disorders. 85.2% was carrier, of whom 30.7% reported symptoms. A minority (33.2%) recalled receiving information about insurance during pretest counselling. 27.8% anticipated that genetic testing would have consequences for future insurance applications. Two thirds (65.6%) of carriers (applying for life insurance; 18/26(69.2%), or disability insurance; 3/6(50%)) were unconditionally accepted. Qualitative open answer analysis showed both positive and complicated experiences during these insurance applications.

Conclusion: Over a quarter of counselled ARRs expected that genetic testing would influence future voluntary insurance applications. Two thirds of applications were unconditionally accepted, the procedure was however experienced as burdensome in a few cases. Our findings suggest that insurance regulations and potential application difficulties pose barriers for ARRs seeking genetic counselling and predictive testing.

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P25.039.D Community-Oriented, Hospital Level Genetics: A New Approach to Improve Access for Underserved Communities

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Background: Certain populations in Israel are at increased risk for genetic syndromes but have limited access to genetic testing.

Material and Methods: We founded a community-based, pediatric genetics clinic in the Muslim-Arab city of Tayibe, Israel. Children with suspected genetic conditions of consanguineous parents, or families with two or more affected siblings were referred by local staff. The clinic was staffed by a Meir Medical Center (MMC) clinical geneticist and tests were analyzed at the MMC Genetics Institute. Blood samples were collected during initial visit. Tests were publicly funded, with no parental involvement in administrative procedures required. A control group consisted of MMC pediatric genetics clinic patients.

Results: During the clinic's first year, 30 children from 19 families were assessed. No patients were lost to follow-up, compared to 8 (28%) in the MMC group. Average time to receipt of test results was shorter in the Tayibe group and the diagnostic rate was higher, with 27.6% receiving a diagnosis (42.9%, excluding autism cases). Questionnaires from Tayibe clinic families

showed that most parents had previously received recommendations for genetic counseling but did not comply, mostly due to lack of interest.

Conclusion: We believe our first-year experience with the Community-Oriented, Hospital Level Genetics Clinic shows success and promising results of this model, with advantages in almost all parameters, compared to the traditional, hospital-based clinic. Factors such as faster time-to-results, greater family adherence and satisfaction, and zero lost to follow-up rate suggest considering implementing this model for providing genetic services to other underserved populations.

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P25.040.A Genetics First Approach: Expanding the Utility of Genetic Testing and Improve Patient Outcomes

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Introduction: The increasing demand for genetic testing and a global shortage of geneticists has significantly strained healthcare systems worldwide. This highlighted the need for new strategies to increase testing accessibility, reduce wait times, and enhance patient care quality.

Materials and Methods: We implemented a four-step program, "Genetics First," to empower non-geneticist physicians (NGPs) to play an active, initiator role in genetic initiatory role in genetic and molecular diagnosis. The steps included (1) establishing criteria to identify suitable clinical domains, (2) selecting clinical indications within the domain through expert panel review, (3) designing tailored education and workflows for NGPs across indications, and (4) monitoring test outcomes and providing further support for complex cases. The test outcomes were compared between NGPs and clinical geneticists (CGs).

Results: One hundred and fourteen endocrinologists completed the program. During the study, 260 gene panels were performed for monogenic diabetes. NGPs initiated 68% of these tests, contributing to a 107% increase in referral rates. The overall diagnostic yields were 30%, with no significant difference in yield between NGPs and CGs.

Conclusion: This study demonstrates the feasibility and impact of involving NGPs in genetic testing, offering a paradigm shift that could expand access to genetic testing and improve patients' care and clinical outcomes.

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P25.041.B Telegenetics: Opportunities and Perceptions in Bulgaria

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Background: Telegenetics, a specialized branch of telemedicine, utilizes digital communication technologies to provide genetic counseling and services remotely. This approach aims to improve access to genetic expertise, particularly in underserved regions. This study explores the adoption of tele-genetics in Bulgaria, focusing on patients' perceptions, accessibility, and the impact on service delivery.

Material and Methods: A mixed-methods approach was employed, combining quantitative analysis of demographic data from 350 tele-genetic consultations conducted in Bulgaria (March 2022 - December 2024) with qualitative surveys assessing patients attitudes towards remote genetic services. Data was collected from telegenetic consultations facilitated via platforms like Healee, Zoom, and Viber and phone calls, ensuring a diverse participant pool.

Results: The analysis revealed increased accessibility to genetic services in remote areas, reducing travel burdens and associated costs for patients. Among the 350 participants, 97% reported high satisfaction levels, emphasizing the convenience and psychological benefits of tele-genetic consultations. Barriers included technological literacy, regulatory challenges, and concerns about standardized practices.

Conclusion: Telegenetics demonstrated significant potential in improving genetic service delivery, especially in regions with limited access to specialized care. The findings support the hypothesis that telegenetics can bridge healthcare disparities, though challenges like regulatory frameworks and digital proficiency need to be addressed. The Covid-19 pandemic served as a catalyst for adoption but highlighted the necessity for long-term infrastructure and policy development. Telegenetics holds promise for expanding genetic healthcare access in Bulgaria. Future efforts should prioritize policy standardization, infrastructure improvement, and professional training for sustainable integration.

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P25.042.C Art and Genetics: we are not different, we are unique

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Background: Iconodiagnosis is the practice of searching for clinical signs suggestive of diseases in people depicted in artworks. We aim to employ art to teach the general public about genetics and to share the educational potential of iconodiagnosis for the medical profession.

Material and Methods: We collected ~800 artworks depicting subjects affected by genetic diseases and other conditions. The pictures were included in a custom-made database with artistic descriptions and reports of the phenotypical features with HPO terms defined by a multidisciplinary team of geneticists. For each representation, a molecular diagnosis was hypothesised.

Results: We will produce a theatrical show focused on iconodiagnosis. The use of scientific methods and terminology will guarantee complete immersion in the world of genetics while offering spectators the possibility to observe artworks from an entirely different perspective. Moreover, we will organise several workshops: Some will be directed to the general public to teach them about rare diseases, while others will be for students and clinicians to practice their ability to perform a "gestalt" diagnosis, raising the discussion on the represented conditions.

Conclusion: The general public will learn about genetic diseases, while students and experts will discover new ways to train the ability to observe, an essential skill for all clinicians. All participants will rediscover the importance of empathy towards patients, considering not only their clinical condition but also their life experiences. The key message will be that we are all genetically different and perfection does not exist; thus, our differences may be the common meeting point.

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P25.043.D Personalized breast cancer screening acceptability in women population from Navarra

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Background: Scientific evidence showed the necessity of adapting breast cancer screening programs from a "one fits all" perspective to a personalized perspective. An important pillar of the implementation process is women acceptability. One of the objective of NAGENMx project is to investigate women vision towards the implementation of personalised breast cancer screening in Navarra region.

Material and Methods: An online questionnaire survey focused on exploring acceptance of personalized risk based breast cancer screening was conducted, with a general population breast cancer risk group (n = 269) and a high breast cancer risk group (n = 209). Results of the acceptability questions were quantified in the Likert scale and summarized with descriptive statistics.

Results: Both groups showed a high agreement on implementing personalized screening (mean = 4.3; SD = 1.1; mean = 4.0; SD = 1.2) and also on conducting more mammograms if their risk is high,(mean = 4.7; SD = 0.6; mean = 4.5; SD = 0.9). But agreement was lower regarding having less mammograms if their risk was low (mean = 3.0; SD = 1.4; mean = 2.7; SD = 1.4).

Conclusion: There is a positive attitude towards personalized screening implementation and increasing prevention if the risk is higher, but there is a lower acceptance to have less mammograms if breast cancer risk is low. This acceptability is shown to be lower among women with high risk of developing breast cancer or already had breast cancer. This could be due to perceived risk and their personal experience. This study has been extended and additional results showed that education about personalized breast screening improves people understanding increasing acceptability.

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P25.044.A Mainstreaming oncogenetic services in a large HMO setting

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Background: Germline genetic testing is increasingly recognized as crucial for treatment planning in cancer care. In Israel, the national healthcare system covers genetic testing for common BRCA mutations for patients with breast, ovarian, or pancreatic cancers. Additionally, healthy relatives can seek testing for reassurance, surveillance, or preventive measures. This shift has led to a new model of genetic testing, aimed at improving clinical decision-making and enhancing patient management through risk-reduction strategies and tailored therapies The goal is to improve access to oncogenetic services and reduce waiting times for patients and family members.

Material and Methods: Maccabi Healthcare Services (MHS), serves 25% of Israel's population, launched a centralized genetic counseling program to optimize genetic testing access. The program involves five certified genetic counselors who triage testing requests based on personal and family medical histories using a simple questionnaire. Requests are categorized into three groups: high-risk patients with cancer or known mutation in relatives, low-risk healthy individuals with a familial cancer history, and those with unique genetic syndromes requiring face-to-face counseling.

Results: From June 2020 to December 2024, the program reviewed 20,895 questionnaires. Among these, 13.5% were cancer patients, 8% had family members with a mutation, and 66.5% had family history of cancer. Only 6.1% required face-to-face counseling.

Conclusion: This triage system significantly reduced waiting times, enabling faster access to genetic testing and counseling. Ongoing evaluations ensure the program remains efficient and effective in meeting patient needs. This approach demonstrates how centralized genetic counseling can enhance healthcare services and improve patient outcomes.

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P25.045.B Unlocking the Potential of Genetic Screening in Pediatric Cancer: Spanish Expert Consensus on newborn Testing, CANGUR project

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Background: The newborn heel prick test screens for treatable diseases, improving morbidity and mortality through early diagnosis. Next Generation Sequencing technologies could expand screening to include more diseases. This project aims to pre-select pediatric cancer susceptibility genes for discussion and consensus with genetics and oncology professionals, evaluating which genes could be candidates for newborn screening in our healthcare system.

Material and Methods: A committee of experts (N = 12: genetic counselors, pediatric oncologists, geneticists, and adult

oncologists) used Catalonia public Health pediatric cancer gene list (N = 114; 80 hematologic and 34 solid genes) to assess clinical actionability based on three criteria (Phase I): 1) Tumor onset before age 6, 2) Tumor surveillance guidelines for children under 6, 3) Clinical utility assessment. Selected genes will undergo expert-based (N = 55) consensus agreement assessed with DELPHI methodology (Phase II). Gene review material has been developed, and workshops for clinical and ethical debates were organized.

Results: 21 genes and 2 conditions were pre-selected in Phase I: *ALK, APC, BLM, CTR9, DICER1, GATA2, MEN1, NF1, RB1, REST, RET, SDHB, SMARCA4, SMARCB1, SAMD9, SAMD9L, SUFU, TP53, TRIM28, VHL, WT1*, CMMRD *(MLH1, MSH2, MSH6, PMS2)*, and FANCONI ANEMIA *(FANCA, FANCN-PALB2, FANCD1-BRCA2, FANCS-BRCA1)*. This Phase I gene list will be proposed for expert consensus agreement (Phase II).

Conclusion: Genetic testing for pediatric cancer susceptibility within newborn screening is complex and requires a multidisciplinary team's expertise.

C.O.I. Disclosure:

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P25.046.C Rare disease patients' and relatives' perception of the diagnostic journey/health care - a nationwide membership online survey

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Background: Rare diseases Sweden is a national patient organisation for rare disease. Rare diseases Sweden is an umbrella organisation covering 52 specific diagnose organizations, having almost 17000 members.

Method: A member online survey, including questions on their diagnostic journey and health care needs, was sent out in 2021, and resulted in 1443 responses.

Results: These results include a subgroup of 942 members covering 120 different diagnoses. Over half have a rare disease and the other half are caregivers. Slightly more than one third of the include conditions include Intellectual disability, and one in five include motor impairment. Almost half of the conditions include complex health care needs. 23 % have a general onset at congenital/newborn life stage, 13 % as toddler and 4 % at adulthood. For 60 % the age of onset can vary. Over 1/3 were diagnosed in <6 months. Nearly half were diagnosed within a year whereas for 1 in 10 it took over 10 years to get their diagnosis. 443 underwent genetic testing and 335 received a genetic diagnosis. 1 out of 5 have not seen an expert of expert in their rare health condition. The group for whom it took > 10 years to receive a diagnosis, had to a lesser extent been in contact with a specialist in their condition, their healthcare need were less met, and they had less trust in healthcare system and healthcare professionals.

Conclusion: The study provides valuable insight in the self-assessed diagnostic journey and healthcare of individuals with rare diseases.

C.O.I. Disclosure:

C. Ingvoldstad Malmgren: None. **S. Juran:** None. **J. Luckhaus:** None. **M. Johansson Soller:** None.

P25.048.A Pilot study on the preference and comprehension of data visualization in cancer predisposition genetic counselling

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Background: Genetic counseling necessitates effective communication of complex genetic risks for informed decision-making. Data visualization can enhance understanding, improve risk perception, and empower individuals to make informed health choices.

Material and Methods: Participants were recruited from a genetics clinic in Porto, Portugal. Exclusion criteria included being underage, sensory limitations, a prior cancer diagnosis, and employment in the healthcare sector. Educational levels were categorized into three groups. Eligible individuals participated in semi-structured interviews to assess data visualization preference and comprehension.

Results: A sample of 63 participants, stratified by education level (low, medium, high), reviewed five visualization formats: bar graphs, tables, pictograms, circle graphs, and clock graphs. Bar graphs (30.2%) and tables (28.6%) were most preferred and demonstrated the highest interpretation accuracy. Participants with lower education levels favored pictograms and tables, while those with medium and higher education preferred bar graphs, suggesting familiarity with these formats. Clock graphs were least preferred and understood, with only 7.9% selecting them and a comprehension rate of 33.3%.

Conclusion: Bar graphs and tables were generally preferred and understood by our participants. However, preferences varied significantly across different educational levels. Findings suggest the need for tailored visualization strategies to optimize communication and improve risk comprehension among individuals with diverse educational backgrounds.

C.O.I. Disclosure:

D. Nogueira: None. **C. Azevedo Soares:** None.

P25.050.C Empowering Choices: Mapping Tools and Innovations to support NIPT Pre-Test Counseling

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Background: Various tools and interventions have been developed to support pre-test counseling for non-invasive prenatal testing (NIPT), aiming to empower patients as they navigate important reproductive choices. In this review we aim to identify and map the existing tools and interventions to support NIPT pre-

test counseling, we examine their key characteristics and the outcomes of their evaluations.

Material and Methods: A comprehensive literature search is being conducted using PubMed, Web of Science, and CINAHL. Title and abstract screening, full-text review, and data extraction is being performed independently by two reviewers. Key characteristics, including intervention type, format, delivery mode, content focus, stakeholder involvement, interactivity, target population, evaluation metrics, and reported outcomes, are being synthesized descriptively.

Results: We identified a wide range of interventions that have been developed and/or evaluated, including (digital) decision aids, educational videos, group based counseling, training programs for health care professionals, etc. While these interventions demonstrate potential for enhancing comprehension and ensuring informed consent, their success depends on rigorous design, cultural sensitivity, and equitable accessibility. Addressing challenges related to health literacy, language barriers, and integration into existing systems is essential to complement traditional counseling approaches and promote patient-centered care.

Conclusion: Emerging digital interventions hold great promise, but further research is required to ensure they are effective, accessible and tailored to the needs, values, and preferences of diverse stakeholders. This review highlights key gaps and provides recommendations, stressing the importance of collaboration with end-users and comprehensive evaluation to fully realize the potential of evidence-based counseling tools.

C.O.I. Disclosure:

E. Van Steijvoort: None. **H. Peeters:** None.

P25.051.D The evolving landscape of cancer germline genetic testing; how are carriers being identified?

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Background: Germline genetic testing in cancer susceptibility is complex. This study aims to: (1) assess the indications for cancer germline genetic testing, (2) identify the yield of pathogenic variants (PV) for each indication, and (3) quantify PV carriers identified outside traditional criteria.

Material and Methods: Retrospective analysis was conducted using a hereditary cancer unit registry (2019–2024). Testing indications were classified into: (1) traditional criteria-based panel testing (2) mainstream testing for therapeutic purposes (3) predictive cascade testing, (4) confirmation of secondary findings (SF: PV in cancer susceptibility genes during genomic testing), and (5) reflex germline testing of tumoral PV suspicious of germline origin.

Results: Germline testing increased 61% (from 764 tests in 2019 to 1230 tests in 2024). Of the 5703 tests, 63% were based on traditional criteria, 31% on predictive testing, 5% on mainstream testing, and 1.3% on tumour or secondary findings. Overall, 1261 cancer-predisposing PV carriers were identified, 6% (80/1261) of whom were detected outside traditional criteria. Carrier yields were 13% for panel testing, 43% for predictive testing, and 50% for tumour PV reflex testing (Table 1).

Conclusion: Predictive genetic testing encompasses one third of the indications with one of the highest yield. Human and structural resources in germline genetic testing should focus on predictive genetic testing. Tumour testing and secondary findings from genomic analysis represented 6% of PV identification and would not have been identified by other means. These techniques require complex pathways for identification, interpretation and referral.

Indication of germline testing (2019–2024)	# tests performed (N = 5703)		# carriers identified (N = 1261)		Yield of the indication (carriers/ indication)	
	n	%	n	%	n	%
Criteria-based panel testing	3573	63	414	33	414/3573	12
Mainstream panel testing	310	5	41	3	41/310	13
Predictive single testing for a familial PV	1746	31	758	60	758/1746	43
Confirmation of SF from genomic testing	19	0,3	19	2	19/19	100
Reflex testing of tumoral PV	55	1	29	2	29/55	53

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P25.053.B Enhancing genetics education through an undergraduate journal club: a pilot study

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Background: Journal Clubs (JCs) remain underexplored in undergraduate medical education (UME). This study evaluates the effectiveness of a JC implemented at the Department of Molecular and Medical Genetics, Tbilisi State Medical University, for 2nd- and 4th-semester students in the 6-year Medicine program.

Material and Methods: In 2021, a voluntary JC was introduced with the aims of enhancing genetics knowledge, literature analysis skills, and performance across multiple domains among undergraduate students. The JC was promoted through social media, emails, and personal communication. Biweekly meetings involved student presentations on recent articles covering broad topics in genetics and genomics with faculty mentorship. Discussions followed each presentation, and anonymous peer feedback was collected. A mixed-methods approach, including a 13-question Likert-scale survey and face-to-face interviews, was used to evaluate the JC.

Results: A total of 26 students completed the survey. Students overwhelmingly reported a positive JC experience and increased interest in genetics. All students believed JC enhanced their ability to understand and utilize primary literature. The majority felt the JC improved their understanding of biostatistics (75%) and enhanced communication and teamwork skills. All students would recommend JC to peers and they all expressed a desire for JCs to be implemented in other courses.

Conclusion: The undergraduate student-led JC enhanced students' interest in genetics, research analysis skills, and soft skills such as communication and teamwork. Expanding JC

initiatives could provide educational benefits for undergraduate medical students.

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K. Bregvadze: None. **E. Abzianidze:** None. **E. Kvaratskhelia:** None. **T. Tkemaladze:** None.

P25.054.C Comprehensive Premarital Genetic Screening in Abu Dhabi: A Model for Genetic Disease Prevention

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Background: Premarital genetic screening is a key public health strategy in the UAE, particularly in Abu Dhabi, to reduce the prevalence of genetic disorders and enable informed reproductive decisions. High consanguinity rates in the region, combined with a significant genetic disease burden, underscore the need for a robust screening protocol. This initiative aims to provide couples with insights into genetic risks, thereby supporting informed choices and reducing hereditary disease transmission.

Method: The Abu Dhabi program has expanded the scope of premarital screening in the UAE, expanding from 2 conditions to a comprehensive panel covering 570 genes associated with over 840 autosomal recessive disorders.

Results: A total of 1192 couples underwent premarital genetic testing between October 2022 and September 2024 across 22 clinics in Abu Dhabi. Of these, 159 (13.3%) couples were identified as genetically incompatible. The most frequent observed gene mutation among the tested couples was in *CYP21A2* (13%, n = 21), followed by mutations in the *USH2A* gene, found in 11% (n = 17) of cases, *MEFV* (9%, n = 14), and *ABCA4* (8%, n = 12). Nine (5.6%) of the couples were a carrier of two gene mutations, 1 (0.6%) couple was a carrier of three gene mutations and the remaining (93.8%) were a carrier of one gene mutation.

Conclusion: Abu Dhabi's comprehensive premarital genetic screening program highlights the value of a comprehensive approach in reducing hereditary disease prevalence. The program findings underscore the value of premarital genetic screening in culturally similar contexts and its potential to influence future policies in precision health and prevention.

C.O.I. Disclosure:

M. Al Ameri: None. **S. Al Marzooqi:** None. **W. Mohammed:** None. **H. Hasan:** None. **S. Al Hosani:** None.

P25.055.D The evolving scope of professional practice of roles within clinical genomic services

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As genomic medicine evolves, professional roles within specialist services must adapt to meet the increasing complexity of patient care. The Association of genetic nurses and counsellor (AGNC) and Clinical Genetics Soceity (CGS) in the UK collaborated on updating a previous framework builds on previous definitions, refining the scope of practice for *Clinical Geneticists, Genetic Counsellors, Genomic Associates*, and the newly introduced *Genomic Practitioner* role. It aims to enhance clarity, efficiency, and collaboration across the United Kingdom's genomic services.

A key focus of this update is **Mainstreaming**, which expands the role of genomic professionals in supporting non-genetic healthcare providers. This includes clearer responsibilities for providing guidance on referrals, interpreting genomic results, and leading specialist nurse-led mainstreaming teams in areas such as familial hypercholesterolemia and breast cancer screening.The **Research** section has also been strengthened, reflecting the growing involvement of genomic professionals in *gene therapy trials, psychosocial research, and variant interpretation studies*. This highlights the increasing emphasis on evidence-based genetic counselling and patient-centred genomic research.

We present the updated framework, which aims to be **aspirational**, providing a forward-looking vision of how roles can evolve to streamline a cohesive service delivery, improve patient outcomes, and integrate genomic expertise into mainstream healthcare. By clearly delineating responsibilities, this document supports a dynamic, collaborative approach to genomic medicine across the NHS and beyond.

C.O.I. Disclosure:

R. Rizzo: None. **D. Baralle:** None.

P25.057.B Genetic counsellors as agents of change: National transformation of clinical pathways and upskilling of healthcare professionals in genomic testing for Familial Hypercholesterolaemia

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Background: The NHS Long Term Plan set out the ambition to identify 25% of patients with Familial Hypercholesterolaemia (FH) within 5 years. The National FH Transformation Project aimed to identify effective strategies to increase detection of patients with FH. To support mainstreaming of FH testing and to transform FH pathways.

Material and Methods: A Clinical Pathway Initiative process mapped case finding in FH. A competency framework was developed against which educational resources were mapped. The clinical pathway and competency framework was utilised to underpin the content of a pilot FH workshop. A series of workshops were delivered. A pre and post quiz evaluated the educational intervention on the day. The demand for this workshop far outweighed the capacity to deliver. Therefore, a MOOC (Massive Open Online Course) was developed to meet the demand and ensure sustainability of this programme. Evaluation of this programme was determined through completion of an online survey.

Results: The Workshop: 95 delegates attended from a wide range of healthcare professionals. Pre & Post quiz results showed a significant increase in knowledge.The MOOC: 171 registered from 41 countries; 40% UK; 3% North America. The post course survey reported that 88% found the course met their expectations and they were applying this knowledge to their current clinical practice.

Conclusion: This approach to a National Education Programme has established the feasibility of mass online education of healthcare professionals in a disease specific context. Further research is required to explore the impact of this education and training in the clinical mainstream.

C.O.I. Disclosure:

M.S. Watson: None. **D. Leetham:** None.

P25.058.C From Ideas to Impact: The ESHG-Young Committee's Achievements

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Mroczek[10,5], Mathys Wéber[11,5], Nelson Martins[12,5], Juliana Miranda Cerqueira[13,14,5], European Society of Human Genetics - Young Committee[15]

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Background: ESHG-Young (ESHG-Y) is now in its sixth year and continues to represent young researchers in Human Genetics across Europe. By fostering connections and spearheading educational and professional development initiatives, ESHG-Y has grown into a cornerstone for empowering future leaders in genetics.

Methods: ESHG-Y representatives actively serve in the ESHG-SPC, shaping platform and plenary sessions at the annual ESHG conference. We are also involved in EduComm, Policy and Ethics Committee, and ERN-Ithaca, promoting connections with several organisations under ESHG. The committee maintains strong partnerships with the African Society of Human Genetics (AfSHG), American Society of Human Genetics, Human Genetics Society of Australasia, and several national Young Geneticists Societies (YGS) from European countries.

Results: In 2024, the ESHG-Y organized several symposia, educational sessions, and workshops at the annual ESHG conference. It was instrumental in the 34th European Dysmorphology Meeting and a webinar on Non-Invasive Fetal Autopsy in collaboration with ERN-Ithaca. Collaboration with AfSHG and Jackson Laboratory (US) led to a Functional Genomics webinar.We published our commentary 'Termination of pregnancy and laws in Europe' in EJHG, describing insights from the corresponding ESHG 2023 workshop in Glasgow. Additionally, the committee formed meaningful connections with seven YGS in Europe.

Conclusion: The ESHG-Y marks 2025 as an election year for a new board after completing its first revised full term (2022–25), marked by impactful contributions. We remain dedicated to fostering innovation, international collaboration, and developing future leaders in Human Genetics.

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P25.059.D How should polygenic risk scores be used for cardiovascular disease prevention? A multi-stakeholder qualitative exploration of implementation scenarios and challenges

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Background: Combining polygenic risk scores (PRS) with clinical risk factors into a comprehensive risk profile (CRP) has the potential to improve risk stratification and enable personalized cardiovascular disease (CVD) prevention. However, implementation challenges persist, including defining clinically actionable thresholds, communicating personalized risk, promoting behavior change, structuring follow-up, mapping stakeholder priorities, and addressing ethical concerns. Overcoming these hurdles is vital for translating CRPs into effective clinical practice.

Methods: Within the GOALL project and the TeamPerMed consortium, we developed four implementation scenarios for CRPs in CVD prevention to encourage multidisciplinary discussion and collaboration. Using qualitative research, we will explore stakeholder perspectives on the feasibility, desirability, and acceptability of CRPs across various life stages. These insights will inform the design of future CVD prevention strategies and pilot implementation programs.

Results: By the ESHG conference, we will have developed a preliminary framework for the use of CRPs in CVD prevention. This framework will define clinically actionable thresholds, best practices for personalized risk communication, strategies to use CRPs to promote behavior change, and optimal follow-up monitoring structures. Future qualitative research will refine these elements by incorporating a diverse set of stakeholder views.

Conclusion: This study lays the groundwork for integrating polygenic risk scores with clinical risk factors into CRPs for CVD prevention. By systematically addressing the most pressing implementation barriers through qualitative research, our work will advance personalized cardiovascular risk prediction and prevention. Ultimately, our findings will inform future clinical trials and facilitate the integration of genomic and clinical data into routine healthcare applications.

C.O.I. Disclosure:

D. Vermazeren: None. **J. van Rooij:** None. **A. Uitterlinden:** None.

P25.060.A Challenges in variant reclassification: lessons from four clinical cases and the genetic counsellor's role

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Background: Categorizing genetic variants is challenging, and reclassification is common. Studies show that increasing uncertainty leads to heightened anxiety, mistrust, and confusion. We present 4 cases where reclassification impacted patient and family diagnoses and care.

Material and Methods:

Results: PATIENT A) A 61-year-old patient with familial hypertrophic cardiomyopathy (HCM) and a heterozygous, likely pathogenic NM_001018005.1 c.109A>G variant in *TPM1*, which co-segregated with HCM in the family. Consequences: Non-carrier family members were excluded from follow-up, and one relative pursued preimplantation genetic testing. Reclassification: Variant of uncertain significance (VUS) (PM2, PP1, BP4).

PATIENT B) A 36-year-old patient with MODY and a heterozygous NM_000545.6 c.-160_-154dup likely pathogenic variant in *HNF1A*. Consequences: Cascade testing and endocrinological follow-up for the carrier daughter. Reclassification: benign (BA1, BP5).

PATIENT C) A 79-year-old patient with hypermobility and aortic dilation with a heterozygous, likely pathogenic

NM_000138.4:c.370A>G variant in *FBN1*. Consequences: Cascade testing for the daughter and cardiological follow-up. Reclassification: VUS (PM2, BP4).

PATIENT D) A 39-year-old patient with arrhythmogenic right ventricular dysplasia and two heterozygous pathogenic variants: NM_198056.3: c.5902_5905dupATCT in *SCN5A* and NM_000256.3: c.667G>A in *MYBPC3*. Consequences: Cascade testing, inclusion on cardiological follow-up, and reproductive implications. Reclassification: SCN5A: VUS (PM2 + PVS1). MYBPC3: Likely benign (BS1).

Conclusion: These cases underscore the clinical significance of variant reclassification. Addressing this possibility during genetic counselling—including for pathogenic and likely pathogenic variants—can help reduce emotional distress and enhance patient preparedness. Collaboration with healthcare providers is essential, as reclassification directly impacts diagnosis and follow-up care for both patients and their families.

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P25.061.B The (long) road to licensure

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The profession of genetic counselling, established over 50 years ago, has been present in Iceland since 2006 when the first MSc-trained genetic counsellor started working there. Currently, there are three qualified genetic counsellors at Landspitali University Hospital in Reykjavik, with two more expected to graduate and join the Icelandic GC team by 2027.Efforts to establish professional licensure were first made in 2010. They were denied. In 2022, a new application was sent, supported by the Directorate of Health and medical geneticists at Landspitali.In May 2024, the Icelandic Ministry of Health agreed to the application and introduced a regulation defining the profession's scope, including educational requirements, rights, responsibilities, and employment conditions. To qualify for licensure, candidates must hold a minimum of a two-year master's degree in genetic counselling from an accredited institution, aligning with European and international standards, including those set by the European Society of Human Genetics and the American Society of Human Genetics. The regulation also applies to professionals trained outside the EEA and Switzerland, allowing licensure based on equivalent qualifications.The regulation emphasizes the importance of professional ethics, ongoing expertise, and compassionate, respectful patient care. It aims to standardize genetic counselling in Iceland, ensuring high professional standards, ethical conduct, and the protection of patient rights. Following the regulation and in line with Icelandic law, eligible GCs could now apply for licensure and by October 2024, all three qualified genetic counsellors in Iceland had received their licenses.

C.O.I. Disclosure:

V.F. Stefansdottir: None. **E.T. Thorolfsdottir:** None. **H.B. Hognason:** None.

P25.062.C Transition Care for Adolescents with Hereditary Cancer in Singapore: A Foundation for Future Work

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Background: Hereditary cancer syndromes may require surveillance and management beginning in childhood or early adolescence. Upon reaching adulthood, a transition to adult clinics becomes necessary. Literature on transition care highlights the anxiety experienced by patients as they navigate changes in the healthcare setting. However, there is limited literature on transition of care for adolescents with hereditary cancer syndromes. The Cancer Genetics Service in National Cancer Centre Singapore (NCCS) manages patients with hereditary cancer syndromes through virtual multidisciplinary clinics, such as a specialised 'GenoDerm' clinic for hereditary cancer conditions with cutaneous manifestations. However, there is absence of a structured transition care program for adolescents with other hereditary cancer syndromes.

Materials and Methods: A retrospective medical record review was conducted to evaluate the existing transition clinic to enhance care for adolescents with hereditary cancer syndromes.

Results: During the five-year period from 2020 to 2024, twenty-nine adolescents (mean age 19.8 ± 2.2 years, equal gender distribution) were referred from their paediatric specialties to NCCS for genetic counselling and further management. Majority of the patients (19/29, 65.5%) had Neurofibromatosis Type 1 (NF1). The remaining 34.5% had different hereditary cancer syndromes, with 30.0% of this group lost to follow-up, suggesting a gap in the current transition system for non-NF1 patients.

Conclusion: Transition care is critical in ensuring continuity of care, as well as empowering adolescents to take charge of their health. Further work is needed to improve and strengthen the transition process for adolescents with hereditary cancer in Singapore, particularly for those without NF1.

C.O.I. Disclosure:

T.D. Scriven: None. **J. Chiang:** None. **S. Li:** None. **J. Ngeow:** None.

P25.063.D Detection of carriers of hereditary diseases among healthy individuals: basis for expanded genetic screening

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Background. Knowledge of carrier status for mutations associated with hereditary diseases enables couples to make informed decisions about family planning. Population-level carrier screening provides valuable insights into prevalence of genetic diseases in a specific region or among particular groups.

Material and Method. DNA from blood leukocytes was isolated using a salting-out method. A carrier sequencing panel of 450 genes was performed at Invitae Laboratory on 24 couples from the Lviv Region of Ukraine.

Results: NGS testing of 48 individuals showed that 16 were carriers of one hereditary disease (HD), 15 of two, 5 of three. Additionally, two individuals were carriers of four HD, one was a carrier of five, and one - of six. The most commonly detected mutations were in the *HFE* gene (25%), *GALT* gene (10.4%), *DHCR7* (6.25%), *GJB2* gene (6.25%) *CFTR* gene (4.16%), *PAH* gene (4.16%), *AIRE* gene (4.16%), and *GAA* gene (4.16%). Pathogenic variants of *CYP21A2, SMN1, 1HPS1, CLN3, ATP7B, ETFA, G6PC, NAGLU, CLRN1, CNGB3, SLC22A5, NR2E3, ETFA* were also detected. Two matching pairs were found. In the first couple, both partners are carriers of the *CFTR* gene variants, the second couple - of the *DHCR7* gene and were classified as high risk.

Conclusions: A significant part of the tested individuals (66.7%) were carriers of two or more hereditary diseases, which indicates a high prevalence of pathological gene variants in this sample. Such a distribution of mutations may indicate the need for more expanded genetic screening to identify carriers of key HD among healthy individuals.

C.O.I. Disclosure:

M. Tyrkus: None. **I. Shymanska:** None. **Y. Shargorodska:** None. **S.T. Sobechko:** None. **D. Zastavna:** None.

P25.064.A A comparison between monosomy X and mosaic Turner syndrome- What is the appropriate genetic counseling in each case?

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Background: Genetic counseling when a fetus is found to have a mosaic chromosomal anomaly is challenging, especially when the diagnosis is Mosaic Turner Syndrome (MTS). We studied the clinical data of fetuses and individuals found to have full monosomy X (45,X) or MTS from the cytogenetic and microarray labs in Shaare Zedek Medical Center, in order to better understand the phenotypes, for appropriate genetic counseling.

Material and Methods: We reviewed all cases found to have 45,X or MTS in our labs, between the years 2009–2024. We checked the referral reasons, type of sample tested (blood or prenatal sample) and age at diagnosis.

Results: 57 cases of 45,X and 69 cases of MTS were diagnosed. Of the 57 cases of full monosomy X, 44 (77%) were diagnosed prenatally. The referral reasons were mainly missed abortion and IUFD. Only 13 cases (23%) were diagnosed postnatally, mainly at a very young age. Of the 69 cases of MTS, 9 were excluded due to complex karyotypes. 55/60 (92%) were diagnosed posnatally and Only 5/60 (8%) were diagnosed prenatally. The main referral reasons were recurrent miscarriages (28/60, 47%), infertility (13/60, 22%) and short stature (6/60,10%).

Conclusion: Our data show that most cases of full monosomy X were diagnosed prenatally, tested for severe phenotypes, while most cases of MTS were diagnosed in adult age, with a much milder phenotype. Our observations suggest that the genetic counseling should be different for full monosomy X Vs. MTS.

C.O.I. Disclosure:

A. Ben-Yehuda: None. **O. Weiss:** None. **O. Lobel:** None. **S. Zeligson:** None. **R. Segel:** None.

P25.065.B Improving Patient-Centered Collaboration and Driving Innovation in Rare Disease Care: Insights from the ERN ITHACA 2024 Patient Advisory Board Workshop, Buccarest 2024

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Background and Objectives: The ERN ITHACA Patient Advisory Board meeting in Bucharest on 12 December 2024 brought together 60 participants, including ePAGs, clinicians, and online attendees. The event aimed to foster collaboration across the rare disease community by sharing best practices, exploring patient journeys, and promoting interdisciplinary teamwork. Presentations focused on innovative strategies in rare disease management, including patient-led guidelines, multidisciplinary clinic models, and novel data collection approaches.

Material and Methods: The workshop was structured into two main components. First, participants learned from each other through presentations and flash talks, showcasing best practices such as care coordination initiatives and global registries. Topics also included patient-led guidelines and data management strategies to enhance research. The second component, "The Patient Journey," addressed challenges like diagnosis, grief, and mental health, featuring testimonies and group discussions. Parallel working groups explored family resilience and grief management strategies, promoting dynamic exchanges between patients, families, and professionals.

Results and Impact: Feedback from participants revealed high satisfaction, with an overall rating of 4.68/5. Attendees appreciated actionable insights from the best practice sharing sessions (4.57/5) and the emotional depth of the patient journey discussions (4.68/5). The workshop successfully promoted patient-centered care and interdisciplinary collaboration. Suggestions for improvement included adding more interactive exercises, diversifying topics, and incorporating additional patient stories.

Conclusion: This event highlighted the importance of inclusive, collaborative initiatives and set the foundation for future workshops, including the next Patient Workshop in Bergen, Norway (December 2025), focusing on global care perspectives.

C.O.I. Disclosure:

A. Hugon: None. **D. Dan:** None. **T. Zdolšek Draksler:** None.

P25.066.C The Orphanet Newborn Screening Bibliographical Knowledgebase

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Background: Newborn screening (NBS) is a cornerstone public health measure aimed at early identification of rare, often genetic, conditions during the neonatal period aiming to implement appropriate measures as early as possible to prevent or limit the negative consequences of these diseases on children's health. The Rare 2030 project, commissioned by the European Parliament, emphasized the need for harmonized NBS strategies across Europe to enhance the diagnosis of rare diseases. Key recommendations included sharing health technology assessment data and best practices to support informed and transparent decision-making.

Material and Methods: In response, Orphanet launched an open access NBS library in 2023, designed to centralize and disseminate knowledge in this field. This resource, available in English and French, is a comprehensive bibliographical database including 823 articles so far dating back to 2008, annotated with the corresponding disease ORPHAcode to facilitate retrieval of information. The library is updated monthly through a systematic literature survey including the one transmitted by French High Authority for Health (HAS) and is supported by AFM-Téléthon.

Results: Our study will show the evolution over the years of the number and distribution of publications between the different categories of the library (global scientific, epidemiological, social, economic and regulatory evidence). A particular focus will also be

made on the growing number of articles relating to the Next-Generation Sequencing (NGS) gene-based screening methods for NBS.

Conclusion: By fostering knowledge-sharing and promoting evidence-based practices, the Orphanet NBS library is a vital tool contributing to the harmonization of NBS strategies across Europe.

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P25.067.D Non-directiveness in genetic counselling: development of a scoping review protocol

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Background: Genetic counselling (GC) has traditionally adhered to the principle of non-directiveness (ND) to help individuals make decisions that align with their values and circumstances, without steering them toward specific choices. While ND has been the subject of considerable debate, with some commentators even suggesting a departure from the term, it remains relevant to discussions of GC's ethos and practice. We have developed a scoping review protocol following PRISMA and JBI guidelines, aiming to document the concepts, meanings, values, and practices associated with ND in GC.

Material and Methods: Relevant articles were identified through searches in Scopus, Web of Science, PubMed, and PsycInfo in January 2025 using terms such as "genetic counsel* OR geneticist AND non-directiv* OR nondirectiv* OR non directiv* OR neutrality OR autonomy OR patient-cent* OR patient cent* AND genetic test." Inclusion and exclusion criteria followed the Population, Concept, and Context (PCC) framework, covering original peer-reviewed articles in English from 1975 onward, since this was the first published definition of GC (by the American Society of Human Genetics). All research methods were considered, while grey literature was excluded. Commentary and review articles were included to capture diverse ideas, values and research findings on ND in GC.

Results: The search yielded 1151 papers; we will now proceed to duplicate removal, data extraction, and analysis.

Conclusion: This is the first comprehensive literature review examining the concept of ND and how it is used within GC.

C.O.I. Disclosure:

A.F. Couto: None. **M. Barbosa:** None. **A.J. Clarke:** None. **L. Sousa:** None. **Á. Mendes:** None.

P25.068.A A new contest dedicated to DNA Day in Georgia: "Art Genome"

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Background: European and American Societies of Human Genetics, as well as many other national societies support DNA Day events all over the world, including annual essay and video contests [1, 2, 3]. Since these contests are open only to students in grades 9–12, the Georgian Society of Medical Genetics an Epigenetics (GSMGE) and Tbilisi State Medical University (TSMU) have partnered in 2023 to launch a new contest, "Art Genome", for university students as part of the activities of DNA Day.

Material and Methods: Biomedical students from Georgian and international programs were eligible to present their works (digital or traditional art). The artworks were relevant to the specified topics. Scoring was based on the following criteria: compliance with the topic of the competition (5 pts); technical capabilities (5 pts); originality (5 pts); total 15 pts.

Results: A total of 70 art works were submitted in 2023 and 2024. First, second, and third place winners were declared through the judging process. Judges were recruited from the active membership of GSMGE and Tbilisi State Academy of Arts. Winners were awarded monetary prizes. All participants received a certificate of participation. In addition, hands-on workshops "Artificial Intelligence in Diagnosis of Rare diseases" and "CRISPR-Cas9 LacZ Gene Editing" were organized for participants and winners.

Conclusion: The Art Genome contest promotes deepening knowledge in the field of genetics, developing interest in science and developing creative thinking.

C.O.I. Disclosure:

E. Kvaratskhelia: None. **T. Tkemaladze:** None. **S. Surmava:** None. **E. Abzianidze:** None.

P25.069.B Benefits of blended learning in medical genetics education

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Background: We introduced blended learning (BL) into medical genetics courses. This transition was challenging, but collaboration with instructional designers allowed to make our discipline a pilot program for Reims Medical School.

Methods: Teaching was overhauled with objectives: 1) to revise the pedagogical scenarization, 2) to encourage students to return to classes. We developed a pathway available on the university's Moodle platform (7 modules, 30 sequences), adopted an asynchronous e-learning and synchronous approaches for lectures and tutorials, respectively.

Results: With BL, we ensured a coherent integration between distance and face-to-face learning and created more concise and varied teaching materials (e.g. short videos, podcasts, flashcards). Short assessments were scheduled after each module on Moodle, and using an audience response system (Wooclap). Students could post questions on a Moodle discussion forum, with responses provided on a weekly basis. A final satisfaction survey is conducted and Moodle learning analytics are analyzed. After two years of implementing this new course, our goals have been met and student attendance has consistently increased.

Conclusion: Our approach addresses demographic challenges: a decrease in the number of teachers and an increase in the number of medical students. Some of these tools are shared with the midwifery and pharmacy curricula. This transformation requires significant time investment for initial design, ongoing maintenance of materials, and responses on the forum. The diversification of teaching materials adds depth to the courses, surpassing the traditional "student handout" approach. The introduction of BL represents an original and ambitious approach, embraced by both teachers and students

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P25.070.C Educational project in medical school for preparation in complex genetic counseling: breaking bad news and anamnesis collection

Chiara Francesca Longo[1], *Giovanni Micheloni*[1], *Roberto Valli*[1], *Annalia Cianflone*[2], *Lucy Costantino*[3], *Fulvio Ferrara*[1], *Elena Mandorino*[3], *Francesco Pasquali*[1], *Lucia Tararà*[1], *Elena Ferioli*[1], *Andrea Moriondo*[1], *Mario Picozzi*[1], *Giovanni Porta*[1]

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Background: Enhanced understanding of human genome, coupled with advancement in sequencing technology has led healthcare professionals towards precision medicine and its four "P" theory (Flores et al., 2013). Currently, patients may be guided by inaccurate information which derived from direct to consumer genetic tests and the management of incidental findings. Therefore a relationship of trust with the geneticist becomes fundamental. Breaking Bad News (BBN) is a complex clinical task that should be recognized as competency in the training of healthcare professionals (Saleh et al., 2022), as well as the integration with ethics consultation to address distress arising from ethical and cultural dilemmas.

Material and Methods: Communication models from a neurocultural perspective and biofeedback serve as advanced tools for managing complex social dynamics, allowing for the identification of areas for improvement from an intergenerational and cross-cultural perspective.Recent studies on feedback in healthcare training based on facial expressiveness (Woods et al., 2024; Singh et al., 2021) could enable an accurate assessment of the effectiveness of doctor-patient communication in the genetic field and provide appropriate communication standards.

Results: We will compare the collected data with the measurement of the trainee's self-awareness to identify areas for improvement and establish a protocol for genetic counseling in various contexts, with a focus on burnout prevention, time optimization, and resource management in patient care.

Conclusion: The proposed training project integrates biofeedback and expressive analysis to enhance BBN skills in healthcare students, optimizing genetic counseling in cross-cultural and intergenerational contexts while preventing professional burnout.

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P26 Ethical, Legal and Psychosocial Aspects in Genetics

P26.001.D Parent perspectives on whole genome sequencing for Autism Spectrum Disorder

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Background: Given the level of genetic heterogeneity associated with ASD, whole genome sequencing (WGS) is a powerful research and diagnostic tool. Due to the potential for secondary findings and the currently limited treatment options available, parent and patient perspectives on the use of WGS in this context are much needed.

Material and Methods: Parents (and qualified children) enrolled in an ASD genomics study were invited to participate in semi-structured interviews about their experiences with WGS. The interviews occurred prior to genomic sequencing (time 1) and after sequencing results were returned (time 2). Eighteen participants were interviewed for time 1 and time 2, respectively. Analytic techniques consistent with interpretive description were then used.

Results: Four themes emerged: contribution to science, hope for treatment, understanding cause and responsibility. The motivation to contribute to science was a prominent theme at both timepoints. By contrast, the hope for treatment identified at time 1 shifted to disappointment at time 2, when it became clear that results would not lead immediately to treatment. Identification of a genetic cause of ASD was associated with relief by some parents, and responsibility by others, depending on how they ascribed blame/blamelessness.

Conclusion: A diagnosis of ASD was an inclusion criterion for the genomic study we recruited from. Though children in this study would have access to standard of care treatments, their parents clearly harboured hope for additional treatment options following WGS. Researchers and clinicians employing WSG need to further reinforce how sequencing is not a panacea for disorders like ASD.

C.O.I. Disclosure:

M.J. Szego: None. **J.A. Anderson:** None. **S.W. Scherer:** None.

P26.002.A Understanding the experience of an uncertain newborn screening result (Award Candidate)

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Background: Whole genome sequencing (WGS) is being incorporated into mainstream healthcare, including newborn screening (NBS). In addition to clinically useful information, WGS produces uncertain results that lack available prognostic information. Research suggests uncertain NBS results have negative psychological impact at a crucial life stage. This study sought to create a theory to better understand why this occurs.

Material and Methods: This qualitative study investigated the experiences of parents of children with Cystic Fibrosis Screen Positive Inconclusive Diagnosis, an example case of an uncertain result from current NBS. The researcher interviewed 16 parents from NHS trusts around England. Data collection and analysis used grounded theory methods to create a transferable theory.

Results: The theory charts parents' experience through four phases of adaptation: 'destruction of the family story'; 'reconstructing the story'; 'reclaiming the story'; and 'withstanding imposed incongruity'. Core to parents' experience is the 'square peg, round hole' paradox: this type of NBS result does not fit well with medical services, communications and understanding that

currently appear unable to accommodate uncertainty. Although uncertain results are not diagnoses, the concept of a diagnosis received undue focus in communication and management of the result, risking unnecessary medicalisation and distress. Parents were negatively affected by experiences, such as interactions with medical services, that disrupted their progress through the phases of adaptation.

Conclusion: The outcomes of the study emphasise the need to modify medical services, communications and understanding in order to healthily accommodate the range of results that will be produced in the genomic era.

C.O.I. Disclosure:

F. Johnson: None. **F. Ulph:** None. **R. Macleod:** None. **K.W. Southern:** None.

P26.004.C Scope, context and implications for practice of familial genomic information: a UK workshop report

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Background: Genomic information is often relevant to more than one family member. This can present conflicting obligations to healthcare professionals (HCPs): balancing patient confidentiality with prevention of harm to another person. In 2020, a case in the UK High Court (ABC vs St George's Healthcare NHS Trust) established that HCPs have a legal 'duty to consider' disclosing information to a relative who is at risk of serious harm. UK professional guidance have long recommended this, but how to apply it in clinical practice is not always clear.

Material and Methods: A workshop was held in November 2023 to identify areas of consensus to inform future professional guidance. 53 participants attended, including patient representatives, HCPs from different specialities, lawyers and academics. Four presentations covered the key clinical, ethical and legal considerations and were followed by a facilitated discussion. A transcript of the workshop was analysed, themes identified and summarised in a report.

Results: Three main themes were identified and will be discussed in more detail: (1) Patient and familial perspectives and considerations; (2) Healthcare professional perspectives and considerations; (3) The role of legal structures.

Conclusion: This workshop clarified the UK legal position and how this aligns with existing professional guidance. A duty to consider relatives may be enacted by using anonymised familial information without specific consent. Resources and further professional guidance are needed to support both HCPs (including those working in non-genetic specialisms) and patients in sharing information. It is crucial to work with patient organisations to co-develop and disseminate these.

C.O.I. Disclosure:

H. Carley: None. **T. Clancy:** None. **S. Weller:** None. **A.M. Lucassen:** None.

P26.005.D Informed Consent: An Unexpected Barrier for Inclusivity in Research on Public Engagement

Isabel R.A. Retel Helmrich[1], *Diewertje Houtman*[1], *Wendy Geuverink*[2], *Joosje Kist*[1], *Rosan Lechner*[1], *Lidewij Henneman*[3], *Martina Cornel*[4], *Sam Riedijk*[1], *De DNA dialogen consortium (The DNA dialogues consortium)*[5]

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Background: In the Dutch DNA Dialogues we aim for inclusive public and stakeholder engagement (PSE) to enable societally aligned policy making for human germline gene editing (HGGE). We engage groups not typically included in PSE, and encounter challenges with obtaining informed consent. In research, the informed consent procedure is intended to safeguard informed decision making and autonomy of participants, through standardized information letters and consent forms. This qualitative study reports on the challenges and aims to explore alternative approaches for inclusive consent procedures.

Materials and methods: We organized over 26 dialogues, which were audio recorded, about HGGE in different settings with diverse publics. We consulted key figures that have familiarity with and are trusted by communities. Together, we identified barriers for inclusivity and based on these co-created alternative approaches regarding language, information provision, options for completion and how and by whom the materials are presented.

Results: The traditional informed consent procedure is a barrier for inclusivity as perceived by researchers and participants alike, due to the logistics being time-consuming and burdensome, complex language, low trust in science or government institutions, and functional disabilities. Accessible language and tailoring based on i.e. design and options for completion is essential. Contact and familiarity with the researcher(s) and key figures should be enabled to facilitate trust. Key figures recommended to provide options for implicit, verbal or anonymous consent.

Conclusion: We argue that traditional consent procedures in research on PSE should be replaced by tailored approaches co-created with key figures, while maintaining voluntariness of participants.

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P26.006.A Perspectives of individuals at risk of genetic frontotemporal dementia on presymptomatic genetic testing

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Background: Presymptomatic genetic testing (PT) for autosomal dominant frontotemporal dementia (FTD) is not taken up as often as initially expected. Few qualitative studies have explored considerations around PT in individuals at risk of genetic FTD. This interview study explores considerations around and experiences with PT in Dutch carriers, non-carriers and individuals at 50% risk of genetic FTD.

Material and Methods: 28 semi-structured qualitative interviews were conducted with Dutch pre-symptomatic confirmed carriers ($n = 11$), individuals at 50% risk ($n = 14$), and confirmed non-carriers ($n = 3$) of genetic FTD about PT, with an average age of 43.7 years. Data were analyzed using inductive thematic analysis.

Results: Reasons in favor of PT included wanting to know and to relieve uncertainty, hoping to test negative, relevance for life planning, informing children's reproductive decision-making, explaining future symptoms, and contributing to the common good. Reasons against PT were fearing psychological impact, fearing effects on employment, insurability or mortgage,

maintaining hope, and no immediate relevance for life planning, partly due to uncertainty surrounding age of onset. Reasons were similar in all groups, but were weighed differently. Decision-making processes were influenced by age, family and friends, and personal experiences with FTD.

Conclusion: Decision-making regarding PT in FTD is highly personal, and seems to be influenced by the individual's tolerance for uncertainty and perceived actionability of genetic information. When supporting individuals at risk of FTD in decision-making, genetic counselors should take into account an individual's characteristics, previous experiences with FTD, the role of family and friends, and clinical heterogeneity.

C.O.I. Disclosure:

C. Graafland: None. **L. Donker-Kaat:** None. **E.M. Bunnik:** None.

P26.007.B Cascade Counselling and Testing. Recommendations of the European Society of Human Genetics

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Background: Cascade testing (CT) is still underused and faces barriers. ESHG contributes to ongoing discussions on how to responsibly optimise informing at-risk relatives and uptake of testing. Recommendations were drafted based on an ethical analysis under what circumstances CT is justified, or its case might be less compelling, based on its proportionality, the actual utility of CT and the judicious use of finite resources.

Material and Methods: ESHG's Policy and Ethics Committee (PEC) and invited experts prepared the draft, ESHG membership consultation was from 25 June until 1 August 2024. Suggestions were integrated where appropriate. The ESHG Board approved the final version by 23 December 2024.

Results: In cases of high risk of serious, avoidable harm a more active approach towards CT is suggested including a more directive approach in counselling, more active support for the proband in passing information to relatives, direct contacting, and allowing the balancing of confidentiality when this is necessary to avoid high risk of serious harm, taking account of national regulations and jurisdictions. In contrast, more caution is advised in more complex cases where the balance of benefits and harms of CT are less clear, such as when penetrance is low, or actionability is limited. This more cautious approach does not call for directivity, direct contact or the relaxing of medical secrecy. The focus, then, shifts to cascade counselling, rather than cascade testing.

Conclusion: Balance between possible benefits and harms is important to assess the proportionality and optimal conditions of CT and cascade counselling.

C.O.I. Disclosure:

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P26.008.C Beyond genomic newborn screening: Using infants' data as a lifetime resource

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Background: Genomic sequencing has the potential to improve newborn screening (NBS) programs by detecting severe childhood-onset conditions, facilitating early intervention and improving health outcomes for children. Yet little research has investigated using genomic NBS data as a lifetime resource.

Material and Methods: To address this, we conducted focus groups with parents of babies who had participated in BabyScreen +, a pilot study that screened 1000 babies in Victoria, Australia for >500 conditions. Focus groups were conducted online and analysed using inductive content analysis.

Results: 32 parents of infants from the BabyScreen+ study participated across eight focus groups. This comprised 29 mothers and 3 fathers; 2 had received a high chance result. Most thought data from genomic NBS should be stored and relooked at throughout the child's life. In terms of potential subsequent analyses, our participants believed certainty that a genetic condition will develop was critical in deciding whether they wanted to receive that information. Although treatability was viewed as important by many, some participants thought they would want to receive information about untreatable and potentially life-limiting conditions because it would give them an opportunity to plan for the future. An age-dependent, staged approach to receiving information was often favoured, although a proportion of parents would want to receive everything as early as possible.

Conclusion: Our findings suggest parents are generally willing for their child's genomic data to be stored and used long-term to improve health outcomes. This knowledge can help inform policy regarding data uses and development of genomic NBS programs.

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P26.009.D Perspectives of Belgian genetic professionals on the reporting of results within reproductive genetic carrier screening

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Background: Since 2019, a reproductive genetic carrier screening (RGCS) offer including more than 1000 genes associated with autosomal recessive and X-linked conditions has been available in Belgium to reproductive partners with a desire to have children. This study sought to investigate the perspectives of Belgian genetic professionals regarding results reporting within RGCS, drawing on their experiences from the past five years.

Material and Methods: Semi-structured interviews were conducted between December 2023 and April 2024 with trained genetic professionals who had direct experience with RGCS. Participants were asked about their views on reporting individual versus couple-based results, incidental findings (IFs), and variants of unknown significance (VUSs) in the context of RGCS. An inductive coding approach was applied to develop an initial codebook which was further refined as additional concepts emerged.

Results: Eighteen trained genetic professionals from six different centres participated. Most favoured couple-based reporting and reporting actionable IFs, but opposed to reporting VUSs in the context of RGCS. Opinions on whether to apply an opt-in or opt-out approach for individual results were mixed, while there was stronger consensus supporting this approach for IFs. Views of participants were mostly shaped by the perceived objective of RGCS, the availability of qualitative pre-test counselling and practical considerations.

Conclusion: This study provides insights into the perspectives of Belgian genetic professionals on the reporting of results within RGCS. While current approaches were broadly endorsed, challenges such as the need to expand counselling capacity were identified to ensure a responsible implementation of RGCS.

C.O.I. Disclosure:

K. Goossens: None. **E. Van Steijvoort:** None. **P. Borry:** None.

P26.010.A A two-decade text mining analysis of language evolution in ESHG conference abstracts

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Background: Language in healthcare and genetics is dynamic and shapes how we interact. Patient organizations and professional bodies have increasingly introduced guidelines to promote inclusivity and sensitivity in language, particularly regarding disability. However, an analysis into how language has evolved in genetics context remains underexplored.

Methods: A text-mining and natural language processing (NLP) approach was applied to 35.745 abstracts accepted at ESHG conference between 2001 and 2021. NLP included data preparation, consolidation and frequency analysis. Specific sets of words related to genetics (e.g., "mutation," "variant"), conditions (e.g., "disease," "disorder"), individuals (e.g., "patient," "proband"), and descriptors (e.g., "diagnosed," "impacted") were included. Linear regression was used to assess temporal changes.

Results: Significant changes in language were observed across all word sets. For genetics set, term "variant" showed increase over time ($p < 1 \times 10^{-3}$), while "mutation" had a non-significant decreasing trend. For conditions set, terms "condition" ($p < 1 \times 10^{-3}$), "syndrome" ($p = 0.017$), "disease" ($p < 1 \times 10^{-3}$), "disorder" ($p < 1 \times 10^{-3}$) and "diagnosis" ($p < 1 \times 10^{-3}$) increased with time, while term "illness" remained constant. For individuals set, terms "patient" ($p < 1 \times 10^{-3}$), "individual" ($p = 0.005$), and "proband" ($p < 1 \times 10^{-3}$) increased, while terms "person", "subject," and "case" remained constant. For descriptor set, increases were observed for "diagnosed" ($p < 1 \times 10^{-3}$) and "impacted" ($p < 1 \times 10^{-3}$), while "affected" and "suffering from" remained constant.

Conclusions: The findings indicate a shift toward more neutral and inclusive language in genetics-related abstracts over the past two decades. These trends reflect broader efforts to align scientific communication with guideline recommendations emphasizing language sensitivity and inclusivity. Results have implications for automated text analysis, abstract preparation, and development of language guidelines.

C.O.I. Disclosure:

A. Ciuca: None. **J. Kasmire:** None. **S. Wynn:** None. **W. Newman:** None. **R. Moldovan:** None.

P26.011.B Multi-cancer early detection based on cell-free tumor DNA: an ethical exploration

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Background: Multi-cancer early detection (MCED) tests are emerging and could be used as population screening. MCED promises early detection of over 50 types of cancer using a blood test and analyzing cell-free tumor DNA. Ethical analysis of MCED is crucial for its responsible development and implementation.

Material and Methods: Based on the ethical core of the screening criteria of the WHO (based on Wilson and Jungner), we provide an ethical exploration of MCED using the principles of 'proportionality', 'respect for autonomy', and 'justice'.

Results: Proportionality requires that the benefits of MCED outweigh its potential harms for participants. The promised benefits of MCED include improvement of cancer screening and prevention or postponement of cancer-related mortality and morbidity. However, study results so far raise questions about the test quality and the diagnostic performance metrics. It is uncertain whether the potential health gains outweigh the false-positives, false-negatives and the accompanying adverse physical, psychological and financial implications. To respect autonomy, individuals should receive understandable and well-balanced information about the test and not experience undue pressure towards using these tests. Additionally, meaningful follow-up actions, such as treatment, should be available. Justice requires that the costs of MCED and follow-up care are balanced against expected health gains and alternative government expenditures, especially pertaining to public health screening. Independent evaluation of MCED, with a critical eye towards commercial interests at play, is crucial.

Conclusion: It is not yet clear whether this screening meets the requirements of proportionality, justice and respect for autonomy. Caution is therefore urgently advised.

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M. Siermann: None. **H. Ismaili M'hamdi:** None. **A. Voogd:** None. **G. de Wert:** None.

P26.012.C Nothing About Us, Without Us Leading, The International Federation for Indigenous Genomics

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Background: "*The genomic health divide must be kept in check and ultimately bridged through equitable economic investment, clinical research, and provision and use of genomic services and technologies globally*." WHO, 2017 This proclamation became the backbone of the Silent Genomes Project, which commenced in April 2018. Although the impetus was the lack of genomic reference data for Canadian Indigenous people noted by clinical geneticists using sequencing for clinical diagnosis, the project scope grew to address genomic inequity at multiple levels. One mechanism to address the genomic inequity was the International Indigenous Genomics Advisory Committee (IIGAC), comprised of international Indigenous experts to provide external guidance on the project, as well as providing insight into international advancements in this area.

Methods: In March of 2023, the IIGAC and additional international Indigenous scholars explored ethically acceptable ways to ensure Indigenous reference data are available for patient care internationally, while being protected from misuse.

Results: The meeting outlined key goals to explore how Indigenous genomic data and background variant databases can be shared internationally in a culturally safe manner to benefit patients with rare diseases, while respecting and integrating FAIR and CARE principles and the operationalization of ethical frameworks, governance processes, and technical infrastructures and consider international genomic and phenotypic data sharing that are Indigenous led.

Conclusion: IFIG aims to share guidance with research granting agencies, research ethics boards and publishers of genomic research so they are able to exert processes to meet their accountability to Indigenous genomic data sovereignty.

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M. Taualii: A. Employment (full or part-time); Significant; Hawaii Permanente Medical Group. **A. Brown:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Illumina. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Illumina. F. Consultant/Advisory Board; Modest; Commonwealth Scientific and Industrial Research Organisation. **N. Caron:** A. Employment (full or part-time); Significant; UBC Northern Medical Program and Dep't of Surgery. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; UBC Centre for Excellence in Indigenous Health. F. Consultant/Advisory Board; Modest; Special Advisor on Indigenous Health; UBC Health and Faculty of Medicine. F. Consultant/Advisory Board; Significant; First Nations Health Authority Chair in Cancer and Wellness at UBC. **M. Hudson:** A. Employment (full or part-time); Significant; University of Waikato. **N. Brown:** A. Employment (full or part-time); Significant; Djanaba Wellbeing. **P. Wilcox:** A. Employment (full or part-time); Significant; University of Otago. **R. Haring:** A. Employment (full or part-time); Significant; Roswell Park Comprehensive Cancer Center. **L. Arbour:** A. Employment (full or part-time); Significant; University of British Columbia. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Genome BC, Genome Canada. **W. Wasserman:** A. Employment (full or part-time); Significant; University of British Columbia. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Genome BC, Genome Canada.

P26.013.D Public perception and willingness to participate in somatic gene therapy first-in-human trials: a Belgian survey study

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Background: Genetic disorders affect millions, yet only 10% of patients receive effective treatment. Gene editing technologies show promise, but first-in-human trials face challenges in defining inclusion criteria and encouraging patient participation. This study addresses a critical gap in understanding public perceptions in Belgium, essential for the responsible development and adoption of gene therapy trials.

Material and Methods: A cross-sectional online survey using convenience sampling was conducted in Belgium with adults (18+) recruited through local pharmacies. The survey included 12 items assessing self-reported knowledge of and willingness to accept gene therapy. To evaluate willingness to accept, hypothetical vignettes were used, which varied by treatment characteristics (e.g., side effects, efficacy, limited evidence), patient age (5, 20, 65 years), and symptomatic status (symptomatic, asymptomatic with uncertain or expected future symptoms). Descriptive statistics summarised all items included in the questionnaire.

Results: Our preliminary study sample consists of 143 participants, of whom 61% had completed higher education, 56% had children, and 86% had heard of gene therapy before. Many participants expressed generally positive attitudes despite unknown long-term effects (79%), side effects (66%), and uncertain effectiveness (77%). However, key barriers included fears of altered identity (36%), external pressure (36%), and skepticism about its novelty (34%). Willingness to accept gene therapy was high across different vignettes, but it declined in scenarios involving asymptomatic patients, regardless of age.

Conclusion: Our initial results indicate a positive trend for gene therapy in symptomatic scenarios, along with a noticeable trend of reluctance toward its preventive use in genetic conditions.

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P26.014.A Receiving results from genomic newborn screening: Findings from the BabyScreen+ study

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Background: Genomic newborn screening (gNBS) can improve health outcomes for infants. More evidence is needed to inform population-scale implementation, however, including benefits and harms to parents. BabyScreen+ piloted gNBS for 1000 newborns in Victoria, Australia. Conditions screened were severe with onset and treatment available by five years-of-age. High chance results were followed by genetic counselling and diagnostic testing. We explored parental experiences of receiving gNBS results.

Material and Methods: In this qualitative study, parents were interviewed at three months post-result. Data were analysed using inductive content analysis, informed by Interpretive Description methodology.

Results: Twenty-one parents participated (18 low chance results; 3 high chance results). Participants described having confidence in the decision to have gNBS and did not report feeling overly anxious while waiting for results. Some participants described being in a "newborn blur" when the result was disclosed, but none felt disclosure should be delayed beyond the newborn period. A low chance result provided participants with psychosocial benefits including reassurance and empowerment. Participants who received high chance results described the disclosure as a "shock" and appreciated prompt genetic counselling. Unexpected subsequent diagnoses in other family members added to the psychosocial impact. Other psychosocial impacts and support needs differed based on the diagnosis. Participants valued the clinical utility of the high chance gNBS results.

Conclusion: Participating in gNBS provides psychosocial benefits to parents who receive a low chance result. The anxiety produced by a high chance result was balanced by its clinical utility and the availability of genetic counselling.

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P26.015.B Psychological support for Huntington's Disease: a Cognitive Behavioural Therapy informed intervention

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Background: Literature is limited in comprehensive evidence supporting the impact of psychological intervention for individuals living with Huntington's Disease (HD). This study aims to investigate the impact of a cognitive behavioural therapy (CBT) for HD. This builds on and extends the *Moving Forward* project of the European Huntington Association.

Methods: The study includes individuals across three categories: individuals at risk of HD, premanifest HD, or with an HD-negative genetic test. The recruitment was facilitated by the Romanian national HD registry. The psychological intervention is CBT-based and consist of eight online individual sessions followed by two group sessions. Assessments was performed at pre-intervention, post-intervention and after the group sessions. The primary outcomes are anxiety, depression and quality of life.

Results: To date, 14 participants have been recruited and study is expected to complete recruitment of 20 participants by end of February. Among the recruited participants, 42% ($n = 6$) have a negative genetic test, 29% ($n = 4$) are at risk, and 29% ($n = 4$) have a positive diagnosis. The cohort is 50% female ($n = 7$). Preliminary results show statistically significant improvements in quality of life ($t = 4.429$, $p = 0.011$), and improvement trends in depression ($t = 1.944$, $p = 0.124$). Anxiety ($t = 0.214$, $p = 0.841$) did not show change.

Conclusion: The project is currently ongoing. Based on the data collected and analysed so far and in a similar previous project conducted by the EHA we expect to see improvement in all psychological outcomes measured. This project will contribute to the current evidence showing the impact of the psychological interventions for individuals impacted by HD.

C.O.I. Disclosure:

M. Radu: None. **S. Trif:** None. **S. Samoila:** None. **F. Julio:** None. **A. Arnesen:** None. **A. Ciuca:** None. **R. Moldovan:** None.

P26.016.C Regional disparities in access to gene therapies in the European Union, United States, Japan, and China

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Background: Understanding the regional differences in approved gene therapies, clinical trial development, and regulatory frameworks is crucial for ensuring equitable access and addressing justice issues in advanced therapeutics. This review aimed to evaluate the differences between the US, EU, Japan, and China and offer policy recommendations to promote harmonization between these countries and regions.

Materials and Methods: A critical review of literature, including peer-reviewed articles, policy documents, national regulatory guidelines, and grey literature was conducted. Sources were selected based on their relevance to accessibility, regulatory disparities, and the selected countries/regions under consideration.

Results: Gene therapy approvals show significant regional disparities, with the U.S. leading with 23 approved therapies, followed by the EU with 16. Few products are accessible worldwide reflecting challenges in obtaining cross-border approvals. Moreover, access is uneven within regions, such as the EU, with high-income countries having better accessibility. High costs and complex reimbursement processes exacerbate these issues, with some products being withdrawn from the market due to pricing disputes. Regulatory differences, such as differing data needs, further delay access in countries, like Japan, where gene therapy products are not available until years after a product is ready for approval. Clinical trial activity mirrors these disparities, with China's growing number of trials potentially reshaping the landscape.

Conclusion: Harmonizing regulations across regions could streamline the approval process for therapies, making them more efficient and reducing disparities. Key solutions include incentivizing cost reductions through streamlined manufacturing, adopting payment models like rebates or value-based payments, and aligning evidence and reimbursement requirements.

C.O.I. Disclosure:

R. Mohan: None. **M. Reckelbus:** None. **P. Borry:** None.

P26.017.D Beyond the test: patient and provider perspectives on secondary findings in Middle Eastern genomic medicine

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Background: The advent of whole-genome sequencing and whole-exome sequencing has introduced secondary findings (SFs) into clinical genomics, raising ethical and practical challenges. This study explores perspectives on SF disclosure among pediatric patients' families and genetic professionals in the Middle East.

Material and Methods: A cross-sectional study surveyed 287 families of pediatric patients who underwent genetic testing and 154 genetic professionals across multiple Middle Eastern sites. Families were interviewed using structured questionnaires to assess their awareness and preferences regarding SF disclosure, while professionals evaluated their perspectives on SF disclosure and familiarity with ACMG recommendations.

Results: Among families, 124 (43.2%) were aware of SFs, and 163 (56.8%) preferred full disclosure. Of these, 153 (93.87%) supported reporting life-threatening but non-preventable conditions. In contrast, 151 (98%) of professionals supported delaying disclosure of adult-onset conditions in pediatric cases. The only significant association identified was between perspectives on the disclosure of life-threatening but preventable conditions ($P < 0.0001$,Fisher's exact test). Additionally, 106 (69%) of professionals reported that if SFs were not disclosed, they would still document them in patient records. The majority (93.5%) of professionals were familiar with ACMG guidelines.

Conclusion: The findings reveal differences in attitudes between genetic professionals and families regarding SF disclosure. Given the variation in individual preferences, genetic professionals should respect patients' beliefs and preferences when discussing SF disclosure. Additionally, as many professionals support delaying the disclosure of adult-onset disorders in pediatric patients, an ethical framework is needed to guide discussions with parents, ensuring informed decision-making while respecting future autonomy.

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P26.019.B Navigating decisions in preimplantation genetic testing: a 15-year perspective of the Dutch national indications committee for preimplantation genetic testing

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Background: Preimplantation genetic testing (PGT) has been available as a reproductive technique to prevent genetic anomalies in offspring for decades. In the Netherlands in 2009, a national indications committee (LIC) was tasked with guiding decisions on its applicability to every new medical indication, with the aim of safeguarding the ethical acceptability of the use of PGT. This paper reflects on the committee's 15-year journey.

Material and Methods: For this study, we analyzed relevant documentation (i.e. letters, reports, minutes) of the LIC, between 2009–2024.

Results: The LIC considered PGT to be acceptable for 134 of 192 (70%) conditions they were consulted for. The criteria 'severity and nature of the disease', 'risk' and 'treatment options' were documented for the majority. Other criteria from the LIC´s decisional framework (e.g. 'additional medical criteria' and 'psychological and moral considerations') were mentioned for a minority of conditions. Only for a few conditions, the severity was explicitly appraised and characterized. There was a modest increase in requests over the years; however, the nature of assessments remained consistent.

Conclusion: In 15 years, the LIC has shaped the application of PGT for a broad variety of genetic conditions. Through its case-by-case approach, guided by a decisional framework, the LIC has been able to assess a complex interplay of disease characteristics, whilst being faced with ambiguity of genetic conditions. The LIC has safeguarded the ethical acceptability of PGT in the Netherlands, and serves as a promising model for a future in which new and rare genetic conditions will continuously be identified.

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V. van der Schoot: None. **R. Kramers:** None. **E. Bunnik:** None.

P26.020.C Moving beyond the mismatch between professional and citizen perspectives in genomics: The curious case of incidental and secondary findings within the Can.Heal project

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Background: When analysing genomic data, clinically relevant findings that exceed the initial aim of sequencing can be identified, both accidentally as incidental findings (IFs) or on purpose as secondary findings (SFs). The evolution toward large genomic data infrastructures will increase the identification of IFs and SFs. Ethical frameworks should be developed to guide the trustworthy management of these findings.

Material and Methods: In the context of the Can.Heal project, a literature review concerning public engagement initiatives on genomics has been conducted, including 45 publications. Subsequently, a stakeholder workshop has been organised with 43 onco-genomic professionals to integrate their perspectives into an ethical framework for genomic data use.

Results: At first sight, the general public and professionals have a different approach to IFs and SFs. Whereas citizens consider the personal impact of additional genomic results in general, professionals specifically focus on the systemic impact of SFs on the healthcare system. However, these different perspectives can be complementary and they may be explained by different levels of genetic literacy and different interpretations of key concepts in genomics. Moreover, beyond the different perspectives of citizens and professionals, shared interests can be identified, such as respect for autonomy, the importance of relatives, and the significance of counselling.

Conclusion: A trustworthy management of IFs and SFs requires a move beyond the disparities between different stakeholders. Identifying underlying and shared values and clarifying key concepts may uncover a common ground on which an ethical framework for genomic data use can be founded.

C.O.I. Disclosure:

M. Saelaert: None. **C. Mayeur:** None. **W. Van Hoof:** None.

P26.022.A DEFIDIAG-DS: Reasons for accessing secondary findings and the consequences of reporting them in 2500 French patients

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Laurine Tempe[24], Claire Kastner[24], Caroline Schluth Bolard[25], Stanislas Lyonnet[26,27], Jean-François Deleuze[28], DEFIDIAG-DS Consortium[29], Marcela Gargiulo[30,31], Catherine Lejeune[4,3], Bénédicte Gerard[25], Hélène Dollfus[1], Perrine Moulinie[1,3], Françoise Robert[10], Christine Binquet[4,3], Laurence Faivre[1,2,3]

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Background: Genome sequencing has revolutionised the diagnosis of rare diseases, but it can also reveal additional findings (AF) (incidental (IF) or secondary findings (SF)).

Methods: The DEFIDIAG-DS study, ancillary to a PFMG pilot project, offered parents of children with Intellectual developmental disorder in order to allow them (excluding minor children) to access to SF ACMG list version 2. This longitudinal mixed-method study, combined questionnaires and semi-structured interviews to explore reasons declining SF, and/or shifts his/her opinion along the consent process and the consequences of receiving SF.

Results: 2,500 parents were offered SF and 85% opted-in. This rate being highly variable between the 13 participating centres (64%–97%). Overall, 65 SF (3%) were returned. 76 parents, who opted out, completed questionnaires and 34 parents who had received SF were interviewed at result delivery and 19 one year later. Over 95% of parents expressed satisfaction with the information provided and the time given to consider it. Following the SF report, over 93% of parents expressed satisfaction due to the medical actionability and the opportunity to inform their family members. While 45% initially reported concerns about the results, this figure decreased to 28% after one year. At one year, 28% of parents reported lifestyle changes, but many adjusted their medical follow-up. 94% shared the results with their families, with positive feedback.

Conclusion: Providing clear information is crucial in the overall process. and Future research should focus on evaluating the clinical utility of SF, particularly in terms of preventing deaths and detecting subclinical conditions.

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P26.023.B Responding to recruitment barriers in community-engaged genetics research

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Background: There is growing consensus on the importance of community-engaged research on the clinical translation of genetic therapies to foster productive relationships and engender trust in research findings. However, the literature has reported it is difficult to recruit and enroll participants.

Material and Methods: Our U.S.-based study is recruiting from ten diverse genetic condition communities to explore perspectives on prenatal gene editing. We designed a systematic approach to optimally facilitate recruitment among these genetic condition communities. We also report on adaptations to recruitment design in response to identified barriers.

Results: We established a dedicated community engagement outreach team to identify community organizations for recruitment partnerships. The outreach team met weekly to strategize across conditions and review contact approaches and response rates. Factors that impacted response included: limited organizational bandwidth, direct communication with organization

leadership and community members, and interest in research partnerships. Response rates were higher for organizations representing less widely known genetic conditions, even though larger organizations had more expansive research recruitment infrastructure. We modified the recruitment design to include outreach to regional branches. In response to organizations stated preferences and needs, we also considered financial contributions, participation in community events, publicizing the research partnership, and following recommended modes of recruitment. We have successfully partnered with organizations for eight genetic conditions and reached enrollment targets for four; engagement is ongoing.

Conclusion: Successful recruitment from genetic condition communities is possible by establishing mutual partnerships with organizations. Partnerships are strengthened when community values are prioritized in recruitment design.

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P26.024.C Forum OncoGenEtica: ethical concerns among Italian healthcare professionals about equitable access to preventive measures for individuals predisposed to cancer

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Background: Modeled on the UK Genethics Forum, Forum OncoGenEtica was established in 2014 to provide Italian healthcare professionals working in cancer genetics and hereditary cancer with opportunities to discuss ethically complex cases. The first three years of activity were described previously. We report here on the cases discussed between 2017 and 2024.

Material and Methods: Quantitative data synthesis and qualitative content analysis of the case reports were performed.

Results: Overall, 15 meetings were held, and 59 cases were discussed. The median number of participants was 35 (18–59), with non-genetics professionals increasing from 5% in 2017 to 20% in 2024. The main ethically relevant issues discussed were: communication of genetic risk in cascade testing; genetic testing for late-onset conditions in minors and young adults; informed consent and genetic counseling in mainstream genetic testing pathways; interpretation and application of privacy legislation; the right to know; the right not to know; value conflicts with colleagues; complexity and uncertainty in returning results. Administrative barriers, financial constraints, and organizational inefficiencies in healthcare institutions, and a fragmented health data system across Italian regions were cross-cutting themes.

Conclusion: Diagnostic approaches to identify individuals with a hereditary susceptibility to cancer continue to expand, providing important opportunities for cancer prevention through early detection, targeted surveillance and risk-reducing surgeries. At the same time, as more non-genetics professionals participate in Forum OncoGenEtica, discussions reveal ethical concerns regarding the capacity of the public Italian healthcare system to ensure timely, appropriate, and equitable access to preventive care for cancer-prone individuals.

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P26.025.D Exploring decision-making and experiences regarding health-related direct-to-consumer genetic testing (DTC-GT) among Dutch consumers

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Background and Objectives: There is a significant research gap in European studies qualitatively assessing the decision-making and experiences of individuals that have undergone health-related direct-to-consumer genetic testing (DTC-GT). We employed an interview-based approach to investigate these elements among Dutch consumers of health-related DTC-GT.

Materials and Methods: We conducted semi-structured, one-on-one interviews with twenty Dutch citizens that underwent health-related DTC-GT in the last ten years to examine their decision-making and experiences therewith. Participants were recruited via diverse sampling methods, including research agencies, social media ads, and snowballing.

Results (preliminary): Participants were often inspired by experiences of previous consumers of DTC-GTs (relatives or acquaintances), discounts, and/or adverse experiences in/with the public healthcare system to undergo DTC-GT. Not all participants considered potential negative consequences of undergoing DTC-GT during decision-making. Actions undertaken by participants influenced by DTC-GT results included consulting regular and alternative healthcare professionals, initiating supplementation of vitamins and other micronutrients, and taking potentially adverse medical actions such as stopping earlier-prescribed medication and refusing advised vaccinations. Testing of participants' children (minors) was also observed. Participants were content with taking a health-related DTC-GT, and advocated for increasing accessibility thereto.

Conclusion (preliminary): Dutch consumers of health-related DTC-GT overall appeared pleased with what DTC-GT yielded them and did not regret their decision. However, we observed several unwanted side-effects of DTC-GT usage, such as inspiring potentially adverse medical decisions, and increasing pressure on the public healthcare system. These side-effects are concerning and not in line with stimulating responsible societal implementation of genetic testing.

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P26.026.A Dutch national consensus-based recommendations for the disclosure of unsolicited findings during clinical genetic testing

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Background: The primary aim of clinical genetic testing is to identify the genetic cause of a patient's condition. With the widespread implementation of exome- or genome-wide tests, the possibility to identify variants that fall outside the scope of the clinical question, so-called unsolicited or incidental findings, arises. How to deal with these findings has been an issue of debate for decades, leading to numerous recommendations and guidelines, leaving the Dutch genetic centers with their nonsynonymous local policies on unsolicited findings until 2021.

Material and Methods: A national working group, consisting of clinical geneticists and clinical laboratory geneticists representing all genetic centers in the Netherlands, discussed various aspects of unsolicited findings in clinical genetic postnatal germline testing, with the aim to compose recommendations on disclosure of such findings. All genetic workflows (targeted and exome or genome wide tests) were taken into account and all types of unsolicited findings were included (single nucleotide, copy number and structural variants).

Results: A national consensus-based guideline, including a flow diagram, has been developed on how to address unsolicited findings during clinical genetic testing. Furthermore, standardized informed consent forms and patient information material were implemented. All genetic centers follow the national policy, but unsolicited findings are locally discussed in multidisciplinary committees.

Conclusion: The Dutch consensus-based recommendations on unsolicited findings meet the needs of stakeholders involved, can be useful when drawing up policy documents and may add to the harmonization and quality assurance of genetic diagnostic testing in Europe.

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P26.027.B Public attitudes towards genomic data sharing in Portugal: Familiarity with genetics and why it matters

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Background: The success of multi-country, data-intensive initiatives like EU's +1 Million Genomes depends largely on political support and investment. However, another significant but often-unheeded factor is public willingness to share genomic data. EU countries that lack understanding of public knowledge and perspectives about genomics risk being inadequately prepared to address emerging ethical and social issues. These issues can undermine public trust and reduce willingness to share data, ultimately threatening the completeness of EU genomic databases. This is the first national-level study of public familiarity with genetics and attitudes toward genomic data sharing in Portugal.

Methods: Cross-sectional study performed on a sub-sample of the global online survey Your DNA, Your Say including 2058 residents in Portugal. Participants were asked about their familiarity with genetics and willingness to share genomic data with: 1) medical doctors; 2) non-profit researchers; and, 3) for-profit researchers. Data were analysed using descriptive and inferential statistics.

Results: Only 41% of participants are familiar with genetics. Participants' willingness to share genomic data varies according to data processing actors. Nevertheless, 73.6% are willing to share data with at least one actor. Participants who are unwilling (10.8%) or uncertain (15.6%) about sharing data are significantly less likely to be familiar with genetics (OR:0.65; 95%CI 0.45–0.92 and OR:0.70; 95%CI 0.52–0.95), compared to those willing (reference group).

Conclusion: Unfamiliarity with genetics is associated with unwillingness to share genomic data. Efforts to promote public knowledge of genetics are crucial to increase public resolve to share genomic data and ensure ethical participation in genomics research.

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P26.029.D Incidental genomic findings in large scale research: using the 3-I framework to reveal policy considerations

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Background: Incidental findings (IF) can be yielded in genomic research that analyzes data from healthy or ill participants. The need for clear IF policies grows along with the increase in international genomic research, data collection and sharing efforts. This study aims to inform policy discussions and decisions about IF.

Material and Methods: We interviewed key stakeholders involved in Canadian, European, or international research projects. We used the 3-I framework for interview design and analysis, which distinguishes interests (i.e. agendas), ideas (i.e. values), and institutions (i.e. policy structures, e.g. laws) as factors that impact policy decisions. We integrated the three bioethical principles into this framework: respect for persons, beneficence, and justice.

Results: Interviewees were from Canada (n = 7) and Europe (n = 4). Different IF policies are followed and practiced. Policy decisions are impacted by varying interests, ideas, and institutions. Prioritization of distinct interests and ideas varies between policies. Key policy considerations are: determining whose interest is prioritized; determining what is of best interest to the participant; determining who is responsible for what when research and healthcare institutional frameworks create tension; determining what is just and a fair distribution of benefits and burdens between individuals and populations; and determining how scarce time and money should be allocated.

Conclusion: Explicating policy considerations can help to further discuss and decide how IF policies will impact not only research participants, but also patients, citizens, professionals, the public and diverse populations. Technologies, cultural values, and laws and regulations evolve over time. Therefore, continuous discussions should be held.

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P26.030.A Leveraging consensus methods to develop reliable outcome measures for responsible data stewardship in the cloud

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Background: Unique ethical, legal, and social issues arise when managing access to large, publicly-funded genomic datasets in the cloud, especially when they are repurposed for research or have value for commercial cloud service providers. As global genomic repositories migrate to cloud-based platforms, we lack understanding of what stewardship outcomes matter most in overseeing access to sensitive, human genomic data in the cloud. Repositories and data stewards require practical, easy-to-use tools to track progress on stewardship outcomes, and enable transparent reporting to prospective genomic data contributors.

Material and Methods: We combined a scoping review of the cloud governance literature for frameworks, guidelines, policies, and best practices with a three-round modified Delphi study to validate core outcomes of responsible stewardship.

Results: Our scoping review of 560 articles yielded 8 stewardship outcome categories- transparency, authentication, auditing, security, sustainability, standardization, consent and compliance, and community engagement- each with measurable outcomes. Nearly 40 international data access committee members, privacy officers, repository managers, and other data management professionals participated in Round 1 of the Delphi to rank the importance and feasibility of the outcomes.

Conclusion: While the Delphi study is in-progress, we expect to generate a set of core stewardship outcomes and develop specific, measurable criteria. Together, the core outcomes + measurable criteria will create a stewardship impact assessment instrument for cloud-based repositories. Further, we expect to determine minimum standards that repositories need to achieve these outcomes. This will assist in more clearly delineating the minimum standards of ethical data stewardship, not only locally but globally.

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P26.031.B Hidden DNA: the unseen risk of misdiagnosis in genetic testing after bone marrow transplantation

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Background: Genetic testing often relies on DNA extracted from peripheral blood. However, unreported bone marrow transplants can lead to misdiagnoses by introducing genetic material unrelated to the patient. In France, approximately 1,500 transplants occur annually, yet many healthcare professionals remain unaware of the implications. This lack of communication can result in diagnostic errors, inappropriate medical management, and psychosocial consequences. In 2023, France introduced its first recommendations for genetic testing on transplant DNA, but official guidelines remain absent.

Materials and Methods: This study investigated genetic test prescriptions using blood-derived DNA from transplant patients. After identifying laboratory discrepancies, we developed a questionnaire to assess healthcare professionals' awareness, practices, and use of alternative sampling methods.

Results: Preliminary responses (n = 59) reveal significant variability in awareness based on professional background. Laboratory-experienced professionals (technicians, geneticists) displayed high knowledge, while clinicians (genetic counselors, non-geneticist physicians, nurses) showed more heterogeneous results.

Conclusion: This study, the first of its kind in France, highlights gaps in awareness and the lack of European guidelines for molecular biology laboratories and prescribers. Our findings emphasize the need for education, communication strategies, and training programs. Ensuring that the correct patient, not the transplant, is analyzed is imperative. One patient may have the genetic results of another person.

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P26.032.C Disparities in access to gene therapy in the European Union: Ethical and regulatory challenges

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Background: Gene therapies offer groundbreaking advancements in medicine, providing potential cures for previously untreatable genetic disorders. However, ensuring equitable access to these therapies presents a critical ethical challenge within the European Union (EU). This review aimed to assess the adequacy of the EU's centralized market authorization framework and alternative pathways, such as the Hospital Exemption (HE) and Compassionate Use Program (CUP), in addressing access disparities.

Material and Methods: We conducted a critical review of the literature, including peer-reviewed articles, policy documents, regulatory guidelines, and grey literature. Sources were selected based on thematic relevance to key ethical challenges: access, affordability, and geographical disparities.

Results: Although the EU's centralized framework guarantees high safety, quality, and efficacy standards, its implementation

reveals substantial barriers. High costs create significant financial challenges for healthcare systems and individuals, disproportionately affecting low-income countries and regions. Geographic disparities, amplified by fragmented national regulations and uneven healthcare infrastructure, limit patient access, particularly in rural areas. Alternative pathways like HE and CUP aim to improve access but are hindered by inconsistent implementation at the national level.

Conclusion: This review highlights the need for a more cohesive and inclusive EU regulatory framework to ensure equitable access to gene therapies. Addressing economic and geographical barriers is important to realize the full potential of these innovations for all EU citizens.

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P26.033.D Psychosocial Impact of Genetic Testing for Hereditary Hearing Loss (HHL): a Perspective Patient-based Survey

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Background: HL is the most common sensory deficit worldwide (approximately 70% non-syndromic and 30% syndromic forms). Genetic etiology is identified in ~half of HL cases and, despite the known benefits of genetic testing, the psychosocial impact of this test need to be studied deepen, especially for syndromic forms where management is extremely difficult.

Material and Methods: We designed a prospective study with two age-based cohorts and two time points: after pre-test counselling (T0) and two weeks after post-test counselling (T1). Validated scales, including the DASS-21 (anxiety/depression), EQ-5D-5L (quality of life), CBCL (behavioural/emotional problems), and CPAS (obsessive-compulsive traits/intolerance of uncertainty), will be administered to participants and families at each time-point.

Results: Results aim to evaluate the psychosocial outcomes of genetic testing in HL, focusing on differences between children and young adults as well as isolated and syndromic HL forms. We hypothesize that parents, particularly when the diagnosis involves a syndromic form of HL (where at the time of diagnosis not all the signs could be present), may experience stress, indirectly affecting the child's well-being and behaviour. Conversely, young adults may exhibit varied psychosocial responses.

Conclusion: Mental health disorders represent an increasing public health concern. Evaluating the psychosocial impact of genetic testing for HL provides insights into patient needs, offers opportunities to enhance care, and allows for the exploration of associations between molecular diagnoses and psychosocial phenotypes, thereby improving the HHL genotype-phenotype correlations. This is especially pertinent with NGS data, which often enables a molecular diagnosis even before symptoms onset.

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P26.034.A Polygenic Risk Scores in the Clinic. A qualitative Study of Healthcare Providers and Researchers' Perspectives on Ethical Challenges

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Background: Substantial research efforts have started worldwide to foster the clinical translation of Polygenic Risk Scores (PRS). Understanding the views of key stakeholders becomes timely to critically inform the socio-ethical debate, impact future health policy, and currently missing guidelines supporting best practices in healthcare contexts.

Material and Methods: We performed 25 in-depth semi-structured interviews investigating the perspectives of European researchers and healthcare providers from different specialties (clinical genetics, oncology, cardiology, psychiatry) on the ethical and social implications related to the prospective clinical use of PRS. Interview transcripts are currently being analysed thematically with the software NVivo. We expect our final analysis and first draft to be ready by April 2025.

Results: Here we report our preliminary findings on participants' perspectives regarding (1) the appropriate use of the technology and (2) key ethical questions in patient care. Under (1) participants identify general principles of appropriate use that should apply across conditions. The appropriateness of PRS uses is also discussed via disease-specific examples at different stages of the clinical pathway (primary and secondary prevention, diagnosis, prognosis). Theme (2) collects views on the interpretation and communication of the technology, and the potential for patients' harm. Finally, participants express their views and uncertainties on the relevance of PRS findings to family members and the acceptability of testing of minors.

Conclusion: At the conference the first author will present the final and complete thematic analysis of the interviews and discuss the implications and limits of the findings in the light of the broader literature.

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P26.036.C Improving the effectiveness of information, consent and non-opposition practice for genetic analysis in France through a transdisciplinary approach: the example of the INFOGENETIC-FR project

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Background: Prior information and consent are essential rights to uphold individual autonomy, a fundamental bioethical principle in health care and research. This is particularly critical in the context of genetic analysis, where methods and technological advancements have increased complexity, making it challenging for professionals to provide clear and understandable information to patients/research participants.France's 2021 bioethics law

addressed these challenges by improving the legal requirements for genetic analyses and refining opt-out procedures. Analysis of the impact of these legal changes needs to be complemented by an study of information, consent and opt-out procedures practices on the ground to capture the full range of issues.

Material and Methods: Here, we present the objectives and methods of the INFOGENETIC-FR project which aims to identify information and consent needs of adult patients/research participants and to analyse professional practices. We bring together legal, ethical, sociological and public health scholars, and use a participatory research methodology to achieve a truly transdisciplinary approach. Starting from an ethical/legal analysis, we analyze, by means of a literature review, qualitative and quantitative analyses, patients and professionals needs, the issues they encounter in practice and the questions raised by research ethics assessment.

Results: We expect to provide information-tools to help health professionals communicate with their patients/research participants.

Conclusion: We promote the active involvement of all stakeholders, an essential step to ensure the production of tools guaranteeing that the rights to information and consent in the evolving field of genetics are fully respected, while providing information tailored to individual needs.

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P26.038.A Assessment of standards of development of core outcome sets for mental health. Implications for pharmacogenetics trials in psychiatry

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Background: There is important uncertainty regarding what represents the most effective outcomes to be measured in mental health trials. Standardization of outcome measures is crucial for ensuring consistency and comparability across trials and diagnoses. The Core Outcome Set—STAndards for Development (COS-STAD) represent the minimum set of recommendations for Core Outcome Set (COS) development initiatives. This study provides a baseline evaluation of COS development standards for mental health to assess their suitability for use in psychiatry pharmacogenetics trials.

Methods: A search of the COMET database identified 113 published and ongoing studies. Seven COS studies were eligible for inclusion. Two independent reviewers assessed the COS against the 12 COS-STAD criteria.

Results: Six COS studies were included in the analysis, covering five different domains as per the DSM-V. None of the studies met all the COS-STAD standards. The number of standards met ranged from 6 to 11, with a median of 8. Five of the 12 criteria were met by all studies (i.e., defining the setting, defining the health condition, defining the population, involvement of healthcare professionals, and involvement of patients) while only one study met the intervention criterion.

Conclusion: This is the first study to assess the application of COS-STAD in mental health, highlighting a significant need for improvement. Key issues identified include the lack of clarity regarding the intervention where COS can be used, inadequate inclusion of patient perspectives, and ambiguity in outcome definitions and language. This assessment is a first step into further developing COS for psychiatry pharmacogenetics trials.

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P26.040.C Towards a responsible implementation of genome wide tests in screening

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Background: The rise of genome wide sequence techniques has made broad DNA analysis and interpretation possible. This allows expansion of screening for (the risk for) diseases. Although this opens many new avenues for preventive medicine, it also comes with substantial challenges. Our research has identified stakeholder-supported requirements for responsible implementation.

Methods: The research consisted of three interconnected parts: 1) describing the normative genomic screening framework; 2) discussing possibilities for genome wide testing in three screening settings (population screening, opportunistic genome screening and direct-to-consumer (DTC) genome screening; 3) conducting a stakeholder analysis to investigate broad perspectives towards genome wide screening and requirements for responsible implementation.

Results: The stakeholder analysis identified three perspectives, also brought forward in the normative analysis: 1) legislation and guidelines on screening play a key role in protecting screening participants; 2) the autonomy of screening participants should be respected as much as possible; 3) secondary findings should be minimalized and the quality of the screening offer should be optimized. The normative analysis further stressed the importance of proportionality (benefits outweighing costs). Acceptance for implementation was highest for the setting of population screening and lowest for DTC genome screening.

Conclusion: Two main conclusions are drawn: 1) a broader analysis and interpretation of the genome is less easy to justify from the perspective of principles of responsible screening compared to targeted screening; 2) responsible implementation of genomewide testing in the context of screening is best guaranteed within the population screening setting and least in the context of DTC genome screening.

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P26.041.D More of the same? Israel's expanded carrier screening for cystic fibrosis

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Background: Cystic fibrosis (CF) is known as the most prevalent life-limiting genetic disease among individuals of European ancestry. Most countries with a high CF prevalence offer CF testing through newborn screening (NBS) to facilitate early diagnosis and treatment and improve health outcome. Israel, however, chose a unique approach to CF testing. It does not offer NBS, but since 2008 it offers population genetic carrier screening (GCS) with public funding. Carrier couples with increased chance for an affected child receive funding for prenatal testing or in vitro fertilization and preimplantation genetic testing. Recently, the Israeli mutation panel for CF dramatically expanded from 18 to over 70 mutations. This study explores the ethical, social and policy-related implications of this expansion, especially with reference to NBS.

Material and Methods: Literature and document-based analysis was conducted.

Results: The Israeli policy focuses on the provision of reproductive options to couples through GCS. However, the expected lower CF birth rates reduce the likelihood of implementing NBS. This arguably shifts the balance between treatment and prevention (through selective reproduction), further towards prevention.

Conclusion: The unlikely prospect of NBS implementation is of ethical relevance as it negatively impacts future individuals with CF who will not benefit from early diagnosis. Since the expanded panel cannot detect all cases due to incomplete coverage of mutations and partial uptake in the population, NBS and GCS have complementary roles. Together they promote both a wider scope of reproductive choices and better outcomes for individuals born with the condition.

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P26.042.A Death anxiety and depression levels of elderly individuals with Alzheimer's disease

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Background: Alzheimer's disease is known as the most common type of Dementia. More than 70 relevant genetic regions are related to this disease. The APOE ε4 variant of the APOE gene is a significant genetic risk factor for Alzheimer's disease. Genetic cases of frontotemporal dementia are mostly caused by mutations in MAPT, C9ORF72 or GRN. BIN1 are also linked to Alzheimer's disease (NIH, 2024). There are 55.2 million people with dementia globally and this number will rise to 139 milyon in 2050 (WHO, 2022).

Material and Methods: In this quantitative study, personal information form, the Geriatric Depression Scale and the Abdel-Khalek Death Anxiety Scale were administered to 378 elderly individuals with mild Alzheimer's and dementia symptoms living in North Cyprus. SPSS 26.0 program and parametric tests were used to analyze the data.

Results: It was determined that the geriatric depression levels of participants between the ages of 65–70 ($X = 2.633$) and of university graduates ($X = 2.711$)were higher than other participants. The death anxiety of female participants is higher than that of male participants. There is no significant relationship between the geriatric depression scale and the Abdel-Khalek death anxiety scale ($r = -.066$; $p > .05$).

Conclusion: During aging period, individuals may have psychological problems such as depression and death anxiety. Identifying these problems and taking the necessary precautions are important to preserve the quality of life of the elderly people.

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P26.043.B THE IMPACT OF GENETIC INNOVATIONS ON PUBLIC HEALTH: A STUDY OF PREVENTIVE HEALTHCARE STRATEGIES

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Genetic innovations, such as gene editing, genomic sequencing, and AI-driven data analysis, are transforming healthcare by enabling early disease detection, prevention, and personalized treatment. This study explores the role of these technologies in improving preventive healthcare, while addressing challenges related to accessibility, privacy, and ethics. The analysis of survey responses and expert interviews reveals broad support for genetic technologies but highlights significant barriers, particularly in equitable access and data privacy concerns. Despite these challenges, genetic innovations are seen as critical for reducing the prevalence of major public health issues, including cancer, diabetes, and heart disease. The study emphasizes the need for strong public policies to ensure responsible use of genetic data, alongside investment in education and healthcare infrastructure to make genetic testing widely accessible. Ethical frameworks are essential to guide the application of gene editing, ensuring it is used safely and equitably. Future research should focus on addressing the gaps in long-term impacts of genetic screening, developing unbiased AI tools, and ensuring inclusivity in genetic research. By overcoming these barriers, genetic innovations can lead to more equitable healthcare and improved public health outcomes globally.

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P26.044.C Impact of the EU IVDR on your clinical genetics laboratory

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Background: The IVDR 2017/746 is the current legislation governing IVDs in the EU market. It has major implications for IVD manufacturers, with significantly expanded requirements for clinical evidence, post-market surveillance, and device traceability. Perhaps less broadly understood is the impact on clinical

laboratories, as it introduces new obligations for in-house devices in healthcare settings, and restricts the use of such devices when a CE-marked alternative is available. This potentially increases the financial burden and pressure on the healthcare system to ensure that devices used within their laboratories comply with this stricter regulatory oversight. We aim to explore the impact of these new rules on clinical genetics laboratories and describe strategies for compliance with EU law.

Material and Methods: Legislation, scientific literature, ISO 15189, other ISO standards, and industry guidelines were reviewed for best practices in the subject matter, including exploring the context of rare diseases and orphan IVDs.

Results: We have summarized the CE-marking process for tests under the IVDR, how to select and implement them in clinical laboratories, and the requirements for demonstrating IVDR compliance of in-house assays. Moreover, we compared requirements for in-house IVDs with requirements for IVDs manufactured on an industrial scale.

Conclusion: The IVDR includes significant obligations, not just for IVD manufacturers but also for clinical laboratories. New requirements and restrictions will also impact laboratories that develop in-house assays or modify commercial assays for their use. Understanding the rules and timeline of their application is critical to enabling laboratories to plan and stay compliant with law.

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